"A COMPARATIVE STUDY OF SMEAR LAYER REMOVAL AND EROSION IN INTRARADICULAR DENTIN WITH THREE IRRIGATING SOLUTIONS: A SCANNING ELECTRON MICROSCOPY EVALUATION"

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CERTIFICATE

This is to certify that **DR.R.SHOBANA,** *post graduate student (2009-2012) from the Department Of Conservative Dentistry and Endodontics, J.K.K.Nataraja Dental College, Komarapalayam, Namakkal District–638183,Tamilnadu has done the dissertation titled* **“A COMPARATIVE STUDY OF SMEAR LAYER REMOVAL AND EROSION IN INTRARADICULAR DENTIN WITH THREE IRRIGATING SOLUTIONS: A SCANNING ELECTRON MICROSCOPY EVALUATION”** *under my direct guidance and supervision in the partial fulfillment of the regulations laid down by* THE TAMIL NADU DR. M.G.R MEDICAL UNIVERSITY, CHENNAI, FOR M.D.S BRANCH – IV CONSERVATIVE DENTISTRY AND ENDODONTICS DEGREE EXAMINATION. It has not been submitted (partial or full) for the award of any other degree or diploma**.**

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CONTENTS

Smear layer associated with endodontic therapy is very thin, amorphous, composed of organic and inorganic substances and covers the prepared canal walls thereby occluding the orifices of the dentinal tubules. It prevents complete adaptation of the obturating materials to the prepared root canal and prevents the penetration of irrigants & intracanal medicaments into the irregularities of the root canal system and dentinal tubules.

The root canal represents a complex structure where the occlusal one third being highly accessible, middle one third fairly accessible and the apical one third being least accessible. During preparation smear layer tends to be formed on the entire root canal space. Removal of the smear layer can be fairly easy in the occlusal and the middle one third whereas in the apical one third it is relatively more difficult.

Smear layer was first reported by **Eick et al in 1970** [29]. They showed that smear layer was made up of particle sizes ranging from less than 0.5 to 1.5µm. They also encompass a thin layer of grinding debris and overall it is 2-5µm thick, extending a few micrometers into the dentinal tubules. **(Brännström.M and Johnson.G et al in 1974)** [16]

Mc Comb and Smith in 1975 [56] were the first to describe the smear layer on the surfaces of instrumented root canals. They suggested that smear layer consisted not only of dentin but also the fragments of odontoblastic processes, pulpal tissue remnants, microorganisms and their byproducts.

Mader et al in 1984 [51] described the smear layer as two components, first the superficial smear layer and second the smear layer material that was packed into the dentinal tubules for a depth of upto 40µm. They concluded that the tubular packing phenomenon was primarily due to the cutting action of rotary and hand instruments. The process of canal preparation can force the components of the smear layer into the dentinal tubules for varying distances and form smear plugs. (**Brännström.M et al 1980)**.[17]

Cengiz et al 1990 [20] proposed that penetration of smear layer into dentinal tubules was by capillary action as a result of adhesive forces between the dentinal tubules and the smear layer material. This capillary action hypothesis possibly explains the packing phenomenon upto depths of 110µm when using surface active agents within the canal during endodontic canal preparation **(Aktener et al 1989).**[1]

Variations in the smear layer have been reported with the type of instrumentation used to prepare the canals. Formation of smear layer is inevitable during root canal instrumentation regardless of the instrument or instrumentation technique used. Canal preparations without the formation of smear layer may be possible by using a non-instrumental hydrodynamic technique (**Lussi et al 1993).**[49]

Microorganisms were first identified as the cause of pulpal and periradicular diseases by Van Leeuwenhoek in 17thcentury **(Miller WD 1894).**[58] Various microbiologic studies have pointed out the presence of intraradicular microorganisms as a vital factor in influencing the rate of success of endodontic therapy. The root canal system & its ramifications are colonized by microorganisms once the tooth is infected. Microorganisms have also been identified in dentinal tubules as far as halfway through the root dentin of infected teeth **(Shovelton DS 1964).**[93] Microorganisms can not only remain viable but also multiply in the smear layer and penetrate into the dentinal tubules**.**

The presence of a smear layer can prevent the penetration of intracanal medicaments into the dentinal tubules and affects the effective adaptation of root canal sealers to canal walls. One of the primary factors affecting the prognosis of endodontic therapy has been the seal created by the filling material against the walls of the canal. Various studies have evaluated the effect of smear layer on the apical and coronal seal.

The smear layer being a loosely adherent structure should be completely removed from the surface of the root canal as it can harbour microorganisms and cause microleakage. It may also prevent effective disinfection of dentinal tubules by physically preventing the irrigants and intracanal medicaments from reaching the dentinal tubules. The ability of the sealer to penetrate into dentinal tubule is also enhanced by smear layer removal**. (White et al 1987).**[112]

Some authors have suggested that maintaining the smear layer may block the dentinal tubules by altering dentinal permeability. (**Safavi et al 1990).**[80] They proposed that the smear layer acts as a barrier to bacterial metabolites preventing the bacterial invasion of the dentinal tubules**. (Diamond & Carrel 1984).**[27]

There has been an enormous amount of debate and research on the merits and demerits of removing the smear layer before root canal obturation and a mid pathway of modifying the smear in a way that it becomes completely resistant to dissolution and disintegration which results in blocking the tubules has been conceptualized. **(A.P. Tikku et al in 2011).**[99]

Various methodologies have been suggested for smear layer removal from within the root canal system. They are primarily chemicals, Sonics, Ultrasonics, and LASERS either individually or in combination with appropriate root canal preparation techniques. **(Violich D R et al 2010).**[108] Various irrigants like sodium hypochlorite, EDTA, MTAD, organic acids have been used alone or in combination to remove the smear layer. Sonic and Ultrasonic activation of all these irrigants has also been attempted. Passive ultrasonic irrigation which is the activation of the irrigant with an ultrasonically activated file or tip that is not used for canal preparation is probably the most established method for irrigant activation. LASERS are also been used to remove smear layer and eliminate residual tissues in the apical third of the root canal.

Efficient irrigant delivery and agitation techniques are a prerequisite for successful outcome of endodontic therapy. **(Gu et al in 2009).**[38] Certain adjunctive therapies primarily aim to improve the removal of viable microorganisms, smear layer and debris from the root canal system viz., ozone delivery system, photo sensitization technique and high electrical impulse technique**. (Pong-Yin Ng-B in 2004).**[71]

Irrigant delivery systems have also evolved over the years. Irrigant volume, type of delivery, the method of agitation and the depth of delivery are important parameters of which the depth and volume have been shown to be important for removal of debris and microorganism, than the method used**. (Howard et al in 2011).**[42]

EDTA has been used as a chelating agent for negotiating difficult and curved canals. More recently it has been used in gel form as a canal lubricant during Ni-Ti rotary instrumentation of the root canals as a protection against instrument separation. EDTA as an irrigating solution has been shown to effectively remove the smear layer **(Violich D R & Chandler N.P in 2010).**[108]

The tetracyclines which include tetracycline hydrochloride, minocycline and doxycycline are effective against a wide range of microorganisms. They also act as calcium chelators and cause demineralization of root dentin **(Bjorvatn et al in 1982).**[12]

Barkhordar R.A in 1997 [9] was the first to use doxycycline hydrochloride in a concentration of 100mg per ml to effectively remove from smear layer from root canals. Various irrigants based on tetracycline with the aim of effectively combating both the smear layer and the microorganisms have been formulated.

Tetracyclines readily attach to dentin and are subsequently released without losing their antibacterial activity. This creates a reservoir of an antibacterial agent which subsequently is released from the dentinal surface in a slow and sustained manner. Tetracycline based root canal formulations like

Biopure MTAD and Tetraclean have been advocated for use as final root canal rinse before obturation.

Electrochemically Activated water (ECA) is produced by passing a dilute saline solution through a flow through electrolyte (FEM) module to generate (by electrochemical energy conversion) environment friendly highly active solutions of anolyte and or catholyte. Electrochemically Activated water (ECA) was first used in drilling industry. They are biocompatible and do not cause any adverse reactions.

Investigations have revealed Electrochemically activated solution to be effective against a variety of microorganisms (**Vipul kumar et al in 2011)**[109] and the ability to efficiently clean the root canal walls. **(Solovyeva et al in 2000)**.[94] Electrochemically Activated water (ECA) is being used for sterilization of endoscopes and dental unit water lines. Studies of Electrochemically Activated water (ECA) using international tests of exposure and toxicology failed to show any harmful effects of occupational exposure. Electrochemically Activated water (ECA) seems to have a great potential for use as an endodontic irrigating solution.

This study aims to compare the smear layer removal ability of Electrochemically activated water (ECA) with MTAD and 17% EDTA when used in specific irrigant protocols.

The effect of removal of smear layer on the diffusion permeability of human roots was evaluated invitro by **Galvan et al in 1994** [32] and they observed a statistically significant difference between the three groups in the study. A decrease in diffusion permeability of root to Tritiated water (^3H_2O) was noted immediately after smear layer removal and the highest permeability was recorded after storage in the deionized water for 2 months. The model used in this study would allow researchers to study the diffusion permeability of a wide range of endodontic medicaments.

The antimicrobial activity of new super oxidized water (Sterilox) was evaluated for the purpose of disinfection of endoscopes by **Selkon J.B. in 1998.**[85] They found that freshly generated Sterilox was found to be highly effective against a variety of organisms for a exposure time of two minutes. This water (Sterilox) was generated by passing a sodium chloride solution over titanium electrodes at 9amps to produce super oxidized water, which had a redox potential of more than 950mv and a pH range of 5-6.5. The main ingredient is hypochlorous acid (HOCl) at a concentration of about 144mg/l and chlorine (cl_2). This product has been tested for occupational exposure of chlorine and was found to be below analytical detection limits. It was also shown to be non-toxic orally and non-irritant to skin and mucous membrane using internationally tested protocols. They observed that in the presence of high organic load the biocidal activity of superoxidised water is much reduced. This could be negated by working the exposure to Sterilox or by using a sufficient large volume of superoxidised water to reduce the organic load to less than 1%. It was found to be effective against B.Subtilus, E.Faecalis, mycobacteria and wide range of other potentially pathogenic microorganisms associated with the procedures. Freshly generated Sterilox was highly and rapidly effective in killing spore.

The cleaning effectiveness of root canal irrigation with electrochemically activated anolyte and catholyte solutions was evaluated by **Solovyeva et al in 2000**.[94] They concluded that irrigation with electrochemically activated solutions cleaned root canal walls and may be an alternative to sodium hypochlorite in root canal therapy. They found that the combination of anolyte and catholyte resulted in improving cleaning particularly in the apical third of the canals.

Rutela WA and Weber DJ in 2001[78] while discussing the new modalities of disinfection and sterilization discuss a new disinfectant ie; superoxide water. They describe the concept of electrolyzing saline and creating a product which is environmentally safe. The commercial adaptation of this product is Sterilox. The main products are hypochlorous acid (HOCl) at a concentration of 144mg/l and free chlorine radicals which are active for 48hrs. It has a pH of 5-6.5 and ORP of greater than 950mv. This solution has also shown to be nontoxic to the biological tissues. This water has been tested against a wide variety of bacteria, fungi, viruses and spores. It is also effective against Enterococcus Faecalis.

Beltz et al in 2003 [11] analyzed the solubilizing action of MTAD (Mixture of Tetracycline isomer an Acid and a Detergent), Sodium hypochlorite and EDTA (Ethylene Diamine Tetra Acetic acid) on bovine pulp and dentin and concluded that sodium hypochlorite removes the organic components of the pulp and dentin effectively. EDTA is capable of removing the organic and inorganic components in dentin and some organic components of pulp. The solubilizing effect of MTAD is near similar to that of EDTA. They also observed that the main difference between the actions of these solutions is the affinity of Doxycycline in MTAD to dentin. Sodium hypochlorite was found to be capable of removing the organic portions of the smear layer derived from dentin and they dissolved greater than 90% of the pulp tissue in concentrations of 2.6%and 5.25%. MTAD and 17% EDTA showed similar ability to dissolve bovine pulp.

Machnick et al in 2003 [50] evaluated the effect of MTAD on flexural strength and modulus of elasticity of dentin and found that there was no significant statistical difference in flexural strength and modulus of elasticity of dentin bars when exposed to saline or MTAD as per clinical protocol. They concluded that MTAD can be used as prescribed for clinical use without affecting the physical properties of dentin. 1.3%sodium hypochlorite for 20minutes followed by a final rinse of MTAD for 5minutes was the clinical protocol followed. The results of this in-vitro study suggest that MTAD possesses most of the positive qualities of an ideal root canal irrigant.

Shahabang S, Torabinejad M in 2003 [87] studied the effect of MTAD on Enterococcus Faecalis contaminated root canals and compared it with that of sodium hypochlorite with and without EDTA. These results showed that the use of 1.3% sodium hypochlorite a root canal irrigant and MTAD as a final rinse was significantly more effective against E-faecalis than other regimens (Fishers exact test). None of the samples treated with MTAD demonstrated bacteria in the dentinal tubules which could possibly be due to presence of an antibiotic effective against E-faecalis as also the presence of a detergent which could possibly aid the penetration of MTAD into dentinal tubules. They concluded that MTAD is an effective final rinse for the eradication of E-faecalis in the root canals.

Shahabang S, Torabinejad et al in 2003 [88] in their in-vitro study of the antimicrobial effect of MTAD and compared it with that of sodium hypochlorite and EDTA. They found that MTAD was as effective as 5.25% sodium hypochlorite and significantly more effective than EDTA ($P<0.0001$). Furthermore MTAD was significantly more effective in killing E-faecalis than sodium hypochlorite when the solutions are diluted ($p<0.0001$). EDTA did not exhibit any antibacterial activity. They concluded that MTAD was a effective solution for use as a irrigating solution to eradicate enterococcus faecalis. The limitation of this study is that it did not account for the penetration ability of the test irrigants into the root canals. They also observed that MTAD has the ability to remove smear layer effectively and had superior bactericidal activity when compared to sodium hypochlorite and EDTA.

The effect of various concentrations of sodium hypochlorite as an intra canal irrigant on the ability of MTAD (Mixture of Tetracycline isomer an Acid and a Detergent) to remove the smear layer from the canal walls was evaluated by **Torabinejad et al in 2003**.[101] MTAD was used as a final rinse, to remove the smear layer. They observed that though MTAD removes most of the smear layer when used as an intracanal irrigant some of the remnants of the organic component of the smear layer remain scattered on the surface of the root canal walls. The effectiveness of MTAD to completely remove the smear layer is enhanced when low concentration of sodium hypochlorite are used as a root canal irrigant before use of MTAD as a final rinse. They suggested the use of 1.3% sodium hypochlorite during instrumentation because of decreased toxicity and adverse reactions and no significant differences were observed between the various concentrations of sodium hypochlorite with MTAD as final rinse in the removal of smear layer.

In a study on the evaluation of a MTAD (Mixture of Tetracycline isomer, an Acid and a Detergent) by **Torabinejad et al in 2003** [102] on smear layer removal when used as a final rinse on the surface of instrumented canals found that the mixture was an effective solution for the removal of the smear layer and does not significantly alter the structure of dentinal tubules when the canals are irrigated with sodium hypochlorite and followed with a final rinse of MTAD. MTAD was also found to be less destructive to the tooth structure when compared with 17% EDTA (Ethylene Diamine Tetra Acetic acid) used as a final irrigant. Cotton wrapped broaches were found to be more effective and less abrasive than similar instruments covered with bristles or foams. They also emphasized the need for correct efficient delivery of irrigating solutions to the apical third of the root canal to enable effective smear layer removal.

Nagayoshi et al in 2004 [65] on an in-vitro study on the efficacy of ozone on the survival and permeability of oral microorganisms observed that ozonated water was effective for killing gram positive and gram negative oral microorganisms and oral Candida albicans in pure culture. The ozonated water exhibited a bactericidal effect on bacteria in plaque biofilm and inhibited the accumulation of dental plaque in-vitro.

Nagayoshi et al in 2004 [64] evaluated the antimicrobial effect of ozonated water on bacteria invading the dentinal tubules and suggested that ozonated water application may be useful for endodontic therapy. They also noted that when irrigated with sonication, the ozonated water had nearly the same antimicrobial activity as 2.5% sodium hypochlorite. Ozonated water was less cytotoxic.

In a comparative study on the demineralization effect of EDTA(Ethylene Diamine Tetra Acetic acid), CDTA(1,2,CyclohexaneDiamine Tetra Acetic acid), EGTA (Ethylene Glycol Tetra Acetic acid) and citric acid on radicular dentin **Galvao et al in 2005** [33] concluded that 1% citric acid solution to be the most effective solution for root dentin calcium ion extraction. Lower concentrations of EDTA and EGTA were found to be more effective than CDTA. 1% EDTA and 1% EGTA had similar demineralization effect on dentin. They also observed that citric acid at neutral pH did not significantly change the calcium content of root dentin. They recommended the combinations of solutions of sodium hypochlorite and decalcifying agents because no single irrigator is capable of removing both the organic pulpal material and predentin as well as demineralizing the inorganic portions of the radicular dentin. They got onto a suggestion that the acidity of these solutions could be removed by final flushing with water and use of calcium hydroxide sealers, and controlling the exposure time.

Hems et al in 2005 [41] evaluated the bactericidal ability of ozone against a strain of E-faecalis and found that it was effective against planktonic E.faecalis cells and those suspended in fluid, but little effect when embedded in biofilms. Its antibacterial efficacy was not comparable to sodium hypochlorite under the test conditions used. This study used ozonated water produced by delivering ozone in air at rate of 5.8cm3 per second to deionized water to provide an aqueous concentration of ozone to 0.68mg per liter.

Martin MV, Gallagher M.A in 2005 [55] investigated the efficacy of superoxide (optident/sterilox) water for the disinfection of dental unit water lines. They used a 14 week trial to access the efficacy of the disinfectant on the dental unit water lines. After treatment with superoxidised water the bacterial count fell to zero at the end of 14 week trial period.

Even after exposure to the superoxidised water, bacterial counts were recorded during the first week of trial which possibly could be due to the release of bacteria from the residual biofilm. After one week the bacterial count reduced to zero which compares well to the other disinfectant systems used for this purpose. No deleterious effects were found from the use of the disinfectant on the units during the 14 week period. Extensive studies on sterilox using standard international tests for exposure and toxicology have failed to show any harmful effects on the operators or dental units.

Sampaio JEC, Campo F P in 2005 [81] studied the smear layer removal after topical application of EDTA (Ethylene Diamine Tetra Acetic acid) plus a detergent, using scanning electron microscope and found that addition of a detergent to EDTA gel did not improve the smear layer removal of the root surface. EDTA gel was effective at smear layer removal of the instrumented surface.

Torabinejad M et al in 2005 [103] compared the post operative discomfort after cleaning and shaping of root canals using two protocols for removal of the smear layer. He compared 5.25% sodium hypochlorite / 17%EDTA (Ethylene Diamine Tetra Acetic acid) with 1.3% Sodium hypochlorite/ MTAD (Mixture of Tetracycline isomer Acid Detergent). The degree of discomfort was recorded on a visual analog scale, after canal preparation. No significant difference was found in the degree of discomfort between the two groups. The group with MTAD started with a higher pain score and they reported with a lower pain score towards the end of the observation period.

Andrea G et al in 2006 [5] evaluated the role of Carisolv and other auxiliary chemical substances on the removal of smear layer in bovine root canals. They found that a combination of solutions of 0.1% sodium hypochlorite, 10% citric acid and normal saline removed the smear layer efficiently compared to carisolv, normal saline and 0.1% sodium hypochlorite either individually or in combination.

In an invitro study on the efficacy of a new brush covered irrigation needle in removing root canal debris was evaluated using a scanning electron microscope by **Al Hadlaq et al in 2006.**[2] They showed that the Navitip treatment was efficient in cleaning the coronal one third of the root. In the middle and apical one thirds the results were not statistically significant between the groups compared. They observed that the apical third was the least effectively cleaned part of the root canal. They also suggested further development of a technique to demonstrate the efficacy of Navitip treatment in the middle and apical thirds before it can be routinely recommended for root canal therapy.

Giardino et al in 2006 [36] compared the surface tension of two antibiotic based root canal irrigants (MTAD and Tetraclean) with the commonly used root canal irrigants. (17% EDTA, Cetrexidin, cetrimide and chlorhexidine), smear clear (17% EDTA plus Tween 80) and 5.25%sodium hypochlorite. Distilled water was used as a reference. Tetraclean had the lowest surface tension. Both MTAD and Tetraclean are capable of removing the smear layer due to the low surface tension which increases the surface area of contact of the irrigant solutions with the dentinal walls and may permit deeper penetration increasing anti-microbial efficacy.

Marques A.A.F et al in 2006 [54] studied the smear layer removal by Scanning electron microscope and chelated calcium ion quantification of three irrigating solutions by atomic absorption spectrometry in an invitro setting and reported that 17%EDTAC(Ethylene Diamine Tetra Acetic acid with cetrimide) and 17%CDTA (1,2,CyclohexaneDiamine Tetra Acetic acid) had significantly less smear layer throughout the canals than 17% EGTA (Ethylene Glycol Tetra Acetic acid). They also showed that EDTAC and CDTA had greater amount of calcium ions compared to EGTA. He also suggested combining both the methodologies of analysis may contribute to understanding how these solutions act in the root canal, and to determine what volume should be used to remove smear layer from all the canal walls. The efficacy of EGTA could be improved by increasing the pH. The smear layer was removed in all thirds by the three irrigating solutions.

Perez H M et al in 2006 [69] compared the effectiveness of different acid irrigating solutions in root canal cleansing after hand and rotary instrumentation in an invitro setting and observed that 2.5% sodium hypochlorite did not remove smear layer or debris and no significant differences in debris were observed between manual and rotary techniques. Acids used for irrigation were 15% citric acid, 15%Ethylene Diamine Tetra Acetic acid, and 5% orthophosphoric acid. However when acid solutions were used with 2.5% sodium hypochlorite alternatively during preparation and 2.5% sodium hypochlorite as a final rinse post instrumentation, there was effective removal of smear layer and debris and no statistically significant differences showed in smear layer removal between techniques.

Iatrogenic staining potential of sodium hypochlorite / EDTA irrigation with MTAD (Mixture of Tetracycline isomer an Acid and a Detergent) as a final rinse was evaluated by **Tay et al in 2006**.[95] This study found red-purple staining of light exposed root treated dentin when root canals were rinsed with 1.3% sodium hypochlorite followed by use of Biopure MTAD as a final rinse. This could possibly lead to reduction of the antimicrobial efficacy of tetracycline. They also observed that the reaction is not an acid base reaction but a redox reaction that is caused by oxidation of MTAD by sodium hypochlorite. The presence of light is also required for the color change observed. They also found this reaction could be prevented by oxidation of reducing agents like glutathione or 10% ascorbic acid to the irrigation protocol before the use of EDTA. However the solution of the reducing agent has to be prepared fresh every time before application.

Tay et al in 2006 [96] evaluated the reduction in anti-microbial substantivity of MTAD after initial sodium hypochlorite irrigation. They observed that within the limits of this study oxidation of MTAD by Sodium hypochlorite resulted in partial loss of antimicrobial substantivity in a manner similar to the peroxidation of tetracycline by reactive oxygen species. They also recommended further studies which included intermediary rinse of distilled water after sodium hypochlorite was irrigated for different duration and volumes to remove the residual sodium hypochlorite which might be trapped within the dentinal tubules. They also reported that prior irrigation with 1.3% sodium hypochlorite before use of EDTA (Ethylene Diamine Tetra Acetic acid) as a final rinse totally removed the smear layer and the dentinal plugs without erosion of intertubular dentin. Smear layer remnants were observed when only MTAD was used as the initial and final rinse.

Davies J.M et al in 2007 [24] evaluated the antimicrobial effects of various endodontic medicaments on Enterococcus faecalis and found that Biopure MTAD was very effective against the organism when compared with dermacyn, sodium hypochlorite and Chlorhexidine. The results were statistically significant. This study did not address the property of substantivity and only evaluated the ability of the endodontic medicaments to inhibit the growth of the microorganisms.

The comparative ability of EDTA (Ethylene Diamine Tetra Acetic acid) solution and EDTA gel to removal smear layer was studied by **Dotto S R et al in 2007** [28] and found that there was no difference between the EDTA solution and 24% EDTA gel formulation

On an invitro study of antimicrobial efficacy of ozonated water, gaseous ozone, sodium hypochlorite and chlorhexidine in infected human root canals **Estrela et al in 2007** [30] concluded that 2.5% sodium hypochlorite, 2%chlorhexidine, ozonated water when irrigated on infected root canals were not sufficient to inactivate E-faecalis.

Ghoddusi J et al in 2007 [35] evaluated the microbial leakage after using MTAD (Mixture of Tetracycline isomer Acid Detergent)as final irrigation. They demonstrated that MTAD was as effective as 17% EDTA (Ethylene Diamine Tetra Acetic acid) in reducing coronal bacterial leakage when used with AH plus and gutta percha. This study used a bacterial leakage test and the results of the present study showed that the use of MTAD as recommended does not adversely affect the seal of gutta percha with the two types of sealers and its effects on bacterial penetration was the same as 17% EDTA.

Giardino et al in 2007 [37] comparatively evaluated the antimicrobial efficacy sodium hypochlorite, MTAD and tetraclean against E-faecalis biofilm. 5.25% sodium hypochlorite seems to be able to remove completely the biofilm organized on the membrane surface. Whereas newer irrigants fail in this action MTAD and tetraclean seems to cause a better action and take 30-60minutes to eliminate biofilms (too long for clinical use). Tetraclean causes a valid reduction in bacteria after 5 minutes. They suggest further studies to understand the correct action and the correct sequence of different irrigants against the bacteria both in planktonic phase organized in biofilm on the surface of the root canal wall or inside the dentinal tubules.

Newberry BM et al in 2007 [66] evaluated the antimicrobial effect of Biopure MTAD on eight strains of Enterococcus faecalis in an invitro setting. A 1.3% sodium hypochlorite/MTAD (five minute exposure) irrigant combination was used. The results showed that the treatment regimen was effective in completely eliminating growth in seven of the eight strains tested. The MIC/MLC tests showed that MTAD inhibited most strains of E-faecalis in 1:8192 times dilution and killed most strains of E-faecalis in 1:512 times dilution. The exposure time to 1.3% sodium hypochlorite was 15 minutes and use of MTAD was as per manufacturers recommendations. Tetraclean had the lowest surface tension. Both MTAD and tetraclean are capable of removing the smear layer, due to low surface tension which increases the surface area of contact of the irrigant solutions with the dentinal walls and may permit deeper penetration, increasing antimicrobial efficacy.

Sharavan et al in 2007 [90] did a systematic review and meta analysis on whether the smear layer removal reduces the leakage of obturated human teeth invitro. They concluded that smear layer removal improves the fluid tight seal of the root canal system whereas other factors such as the obturation technique or the sealer did not produce any significant effects. The dye leakage test was the favourite means of evaluating the effects of smear layer removal.

Sayin et al in 2007 [84] determined the extent of calcium removal on root canal dentin after 17%EDTA (Ethylene Diamine Tetra Acetic acid), 17%EGTA (Ethylene Glycol Tetra Acetic acid), 15%EDTAC (Ethylene Diamine Tetra Acetic acid with cetrimide) and 1% tetracycline hydrochloride treatment with or without the subsequent use of 2.5% sodium hypochlorite in a invitro setting and found that regardless of treatment time all single treatment solutions and combined solution (treatment solution plus subsequent sodium hypochlorite 2.5% solution) removed significantly more calcium than distilled water (control). 17%EDTA and 2.5% sodium hypochlorite resulted in the maximum amount of calcium removal from the root canal dentin. The authors suggested further studies on the effect of such calcium removal on the adhesion of endodontic sealers and adhesive cements. Also the importance is the effect of calcium removal on the micro hardness of dentin.

Tay et al in 2007 [97] in a in vitro study of the microporous, demineralised collagen matrices in radicular dentin created by commonly used calcium depleting endodontic irrigants, observed that it is difficult to simultaneously remove smear layer and render dentinal tubules patent without demineralising dentin with the commonly used smear layer removing endodontic irrigants(EDTA and Biopure MTAD). The presentation of a demineralised collagen matrix might be viewed as a by-product that accompanies the use of calcium depleting irrigants as final rinses and that these collagen matrices have implications in the bonding of sealer to canal walls, distribution of stresses and raises the question of use of remineralising sealers like MTA.

Tizana Giovannone et al in 2007 [100] evaluated the root canal walls after hybrid preparation with Ni-Ti rotary instruments and four different irrigation regimens using scanning electron microscopy and found that none of the techniques used in the study enabled perfect removal of organic and inorganic root canal wall debris. Irrigation with sodium hypochlorite /liquid EDTA was better than sodium hypochlorite /viscous EDTA gel. They found smear free layer alternating with smear covered areas in the same dentinal wall suggesting Ni-Ti instruments exert different pressures on the root canal wall during canal preparation, producing different smear layer thickness which are not completely removed by endodontic irrigants in the thickest parts. They also found that apical third was the area were more debris and smear layer was found. Irrespective of the rotary technique many canals had unprepared areas in the apical third.

Zand V, Rahimi S, Shahi S et al in 2007 [113] investigated the smear layer formation following preparation of root canals using nickel titanium rotary and hand instruments using scanning electron microscope. During instrumentation 5.25% sodium hypochlorite was used as an irrigant and saline was used as a final rinse. They concluded that the rotary instrumentation (Flex master and RaCe) may be better for canal preparation than NiTi hand instrumentation (NiTi K-File instruments) as they left significantly less smear layer in the apical third of the root canal.

Azarpazhooh AS, Limeback H et al in 2008 [7] on their review of application of ozone in dentistry observe that though invitro studies have suggested a promising potential for ozone in dentistry. Clinical studies have not substantiated this and recommend such studies. The effect of ozonated water as an endodontic irrigant has been tested by some authors with regard to its efficacy against E.faecalis and they have found that other irrigants have been more effective. One study has evaluated use of ozone as a gas in the root canal space. There is conflicting evidence on the invitro application on the use of ozone in endodontics. The excellent biocompatibility and ability to be active either as a gas or liquid makes it a ideal agent to be studied and further improved upon.

Khedmat & Shokubinejad et al in 2008 [48] in their comparative study on the efficacy of removal of smear layer by three chelating agents observed that the protocols used in this study were not sufficient to completely remove the smear layer in the apical third of the root canals. They also observed that the addition of surfactants to EDTA did not result in better smear layer removal compared to EDTA used alone.

Mohammadi Z and Shahriari S et al in 2008 [60] compared the antimicrobial substantivity of Biopure MTAD, 2%chlorhexidine and 2.6% sodium hypochlorite in human root dentin in an in-vitro setting and concluded that the substantivity of Biopure MTAD was significantly higher and retained in the root canal for atleast 28 days. sodium hypochlorite displayed no substantivity.

Nogales et al in 2008 [63] on their study of ozone therapy in medicine and dentistry observed that the future of ozone therapy must focus on the establishment of safe and well defined parameters in accordance with randomized controlled trials to determine the precise indications and guidelines. Ozone therapy presents a potential for atraumatic, biologically based treatment for conditions encountered in dental practice. They observed that ozone has been used in endodontics in various forms as ozonated water, ozonated oil and ozone gas. The oxidative power of ozone characterizes it as an efficient antimicrobial and its use in endodontics seems appropriate. It has been used in endodontics as a root canal irrigant and as an intracanal medicament.

Ring et al in 2008 [76] in an in vitro study on the comparison of the effect of endodontic irrigation on cell adherence to root canal dentin, evaluated the effect of 6% sodium hypochlorite, 2% Chlorhexidine gluconate, aquatine endodontic cleanser and Morinda Citrifolia juice in conjunction with EDTA or

MTAD (as different irrigation regimens) on dental pulp stem cell attachment to canal surfaces, which are important to regenerative endodontic procedures. The results of the present study indicate that aquatine EC/ EDTA had the highest number of dental pulp cell attachment closely followed by Morinda Citrifolia/ EDTA. They were the least toxic. Sodium hypochlorite /EDTA, sodium hypochlorite /MTAD, chlorhexidine /EDTA were the most toxic to the dental pulp stem cells. The results also indicated that smear layer did not influence the attachment of stem cells to root canal walls and hence a 100% removal is not necessary for regenerative therapy. They suggested using saline as a final rinse when sodium hypochlorite, chlorhexidine or MTAD are used to promote attachment of dental pulp stem cells to canal walls.

Different sodium hypochlorite (NaOCl) activation schemes were compared by **Al-Jadaa et al in 2009** [3] and observed that sonic activation with a plastic tip was a safe method to irrigate the simulated root canal systems with regards to canal transportation. Passive ultrasonic irrigation was by far superior to sonic irrigant activation with regards to necrotic pulp tissue removal in simulated accessory canals. The ultrasonic activation with nickel titanium files provided the best performance.

Ardizzoni et al in2009 [6] in their in-vitro and ex-vivo study on two antibiotic based root canal irrigants observed that the results of the study strongly support a wider use of these endodontic irrigants in practice. The study compared Tetraclean, Biopure MTAD, and sodium hypochlorite.

Tetraclean and MTAD exhibited a greater antimicrobial efficacy when compared to sodium hypochlorite.

In computational fluid dynamics study of irrigant flow within a prepared root canal using continuous flow rates **Boutisioukis et al in 2009** [14] found that irrigant needles should be placed 1mm from working length to ensure fluid exchange and that turbulent flow of irrigant leads to more efficient irrigant replacement. Irrigant flow rate appears to be highly significant for determining the flow pattern within the root canal and impart displacement apical to the needle tip (side vented). The apical displacement was not satisfactory for any of the flow rates studied

Desai.P et al in 2009 [26] compared the safety of various intracanal irrigation systems. The irrigation systems evaluated were Endovac and macro cannula, Endoactivator, manual irrigation with max-I probe needle, ultrasonic needle irrigation and Rinse-endo. Endo activator extruded significantly less irrigant than manual, ultrasonic and Rinse-endo groups. Endovac did not extrude irrigant after deep intra-canal delivery and suctioning the irrigant from the chamber to full working length. Endoactivator had statistically insignificant minimal amount of irrigant extruded out of the apex when delivering irrigant into pulp chamber, placing the tip into the canal and initiating the sonic energy.

Gregorio et al in 2009 [31] in their in-vitro study of the effect of EDTA, Sonic and Ultrasonic activation on the penetration of sodium hypochlorite into simulated lateral canals and found that sonic and ultrasonic activation resulted in better irrigation of the lateral canals at 4mm and 2mm from working length. Traditional irrigation alone showed significantly less penetration of the irrigant into the lateral canal and was limited to the level of penetration of the needle. The addition of EDTA did not enhance the effect of the irrigant into the lateral canals. They observed that sonic and ultrasonic activation was effective in reaching the irrigants at the apical third of the root canal.

On a review of contemporary irrigant agitation techniques and devices **Gu et al in 2009** [38] observed that efficient irrigant delivery and agitation are pre-requisites for successful endodontic therapy. New agitation devices and techniques which rely on various mechanisms for irrigant transfer, soft tissue debridement and depending on treatment philosophy removal of smear layers have been introduced. These devices are either manual or machine assisted systems. Overall they seem to have resulted in improved canal cleanliness when compared to conventional syringe and needle irrigation. He observes that in-spite of various studies on endodontic irrigation regimens no well controlled clinical study is available in current endodontic literature. He also notes that evidence based studies that attempt to correlate the clinical efficacy of these devices with improved treatment outcomes should be done. Thus the question of whether these devices are really necessary remains unresolved. Also the practicality and the ease of using these devices from the practitioner's point of view need to be evaluated.

Dynamic irrigation has been advocated as a method of canal irrigation due to its simplicity and cost effectiveness though laborious. Understanding these fundamental issues is important for clinical scientists to improve the design and user friendliness of future generations of irrigant agitation systems and for manufacturer's contentions that these systems play a pivotal role in contemporary endodontics.

The effect of different irrigating solutions on bond strength of two root canal filling systems was established by **Hashem A.A.R et al in 2009** [40] in an invitro setting. The Activ GP root canal filling system and gutta percha with AH plus sealer was used with different irrigation protocols. They found that MTAD (Mixture of Tetracycline isomer an Acid and a Detergent) and MTAD/Chlorhexidine adversely affected the bond strength of gutta percha AH plus sealer. Use of Chlorhexidine in combination with MTAD as a final rinse did not enhance the effect of MTAD on the bond strength of the root canal filling material. (Activ GP)

Huth et al in 2009 [43] studied the effect of ozone against endopathogenic microorganisms in root canal biofilm model and found that high concentrated gaseous and aqueous ozone was dose, strain and time dependently effective against the treated microorganisms in suspensions and the biofilm test model. They observed that sodium hypochlorite was the only method that completely eliminated all types of microorganisms.

Mancini et al in 2009 [52] compared the smear layer removal and erosion in apical and intraradicular dentin with three irrigating solutions using SEM (Scanning Electron Microscope) and observed that application of 1ml of MTAD, 17% EDTA, 42% Citric acid or 5.25% sodium hypochlorite for 1minute followed by 3ml of 5.25% sodium hypochlorite is not sufficient to completely remove the smear layer in the apical third of root canals.

Marins JSR, Sassone LM and Ribero DA in 2009 [53] evaluated the capacity of Biopure MTAD to induce genetic damage in-vitro using two different cell lines. The present study indicates that Biopure MTAD induces genetic damage in-vitro, being the most prominent effect observed in murine fibroblasts. Since DNA damage is an important step in events leading from carcinogen exposure to cancer, the results of the present study represent a potential alert to the correct evaluation of the potential health risks associated with these compounds.

Mohammadi Z and Abbot PV et al in 2009 [61] on their review of antimicrobial substantivity of root canal irrigants and medicaments observed that MTAD was efficient in smear layer removal and effective against E.faecalis. When MTAD was applied to 1.3% sodium hypochlorite irrigated dentin its antimicrobial efficacy was reduced possibly by oxidation of MTAD by sodium hypochlorite similar to peroxidation of tetracycline by reactive oxygen species. MTAD was also more effective against E-faecalis when compared with sodium hypochlorite and EDTA. Comparison of 5.25% sodium hypochlorite /EDTA was found to be more effective than 1.3% sodium hypochlorite/Biopure MTAD in terms of antimicrobial efficacy against E-faecalis. The doxycycline part of MTAD was responsible for the substantivity because of its ready attachment to dentin and subsequent release without losing its antimicrobial efficacy.

They observed that the antibacterial substantivity of chlorhexidine extended upto 12 weeks. The presence of dentin, dentin components (hydroxyapatite and collagen) killed microorganisms and inflammatory exudates in the root canal system may reduce or inhibit the antibacterial activity of chlorhexidine and MTAD. The substantivity of MTAD has been shown to extend upto 4 weeks.

Sayin et al in 2009 [84] analyzed the time dependent decalcifying effects of endodontic irrigants with antibacterial properties and observed that MTAD yielded the least significant demineralizing effect in a five minute application. They suggested that whether this effect is also negligible from the viewpoint of intra-radicular and intra-coronal adhesive procedures. This invitro study was done in smear free root canal dentin. 5% sodium hypochlorite resulted in the maximum amount of calcium removal from the root canal dentin at five minutes. They also observed that 2.5% sodium hypochlorite might be less detrimental to root dentin in terms of surface decalcification.

Shahi Shariar et al in 2009 [89] compared the effect of different rotary instruments on smear layer formation using scanning electron microscope and concluded that within the limitations of the study RaCe instruments produced the least smear layer compared to Flex master and profile instruments. They recommended further studies as only normal saline was used as irrigant and either sodium hypochlorite or EDTA were not used.

A scanning electron microscopic evaluation of the type of smear layer produced by new rotary instruments and effectiveness of different combinations of irrigants was done by **Shishir Shety and Suresh Chandra in 2009**.[92] They concluded that the protaper series of the rotary instruments caused the maximum amount of smear followed by profile rotary instruments. The hand instruments caused the least amount of smear layer. They also found that 3% sodium hypochlorite in combination with 15% EDTA was most effective at removing the smear layer.

In their in- vitro study on the effect of sealers on fracture resistance of endodontically treated teeth with and without the removal of smear layer **Vijay Singh et al in 2009** [106] found that there was no significant difference in the fracture resistance of roots regardless of the presence or absence of the smear layer. They also reported that EGTA (Ethylene Glycol Tetra Acetic acid) as a more potent smear layer removal agent compared to normally used EDTA(Ethylene Diamine Tetra Acetic acid) .

In an in-vitro study of the evaluation of the sealing ability of sealers with and without smear layer by **Vijay Singh et al in 2009.**[107] 17%EGTA (Ethylene Glycol Tetra Acetic acid) was found to be better than 17% EDTA (Ethylene Diamine Tetra Acetic acid) in removal of smear layer and did not cause erosion of the dentinal tubules. This allowed the sealer to completely penetrate the dentinal tubules and provide a better seal. Irrigation with only 5% sodium hypochlorite produced the maximum apical leakage.

The efficacy of Navitip irrigation needle in removing post instrumentation canal smear layer debris in curved root canals was evaluated by **Zmener et al in 2009**.[115] In this in-vitro study they concluded that a

Navitip irrigation needle used with 5.25% sodium hypochlorite and 17% EDTA solution with manual brushing as well as the same method augmented by FileEze was the most effective clinical protocol. They postulated that the brush and the intermittent ultrasonics were effective in the removal of smear layer from the apical third of the canals. They also suggest that Navitip being easy to handle, might be a good alternative to ultrasonics and other methods in cleaning the root canal with minimal chance of temperature rise (as in Passive Ultrasonic Irrigation).

The effectiveness of endo activator on smear layer adhered to root canal surface was evaluated by **Al-Obaida et al in 2010** [4] and they found that the endoactivator was superior in its ability to remove the smear layer attached to the root canal walls when compared to the non activated group, which was possibly due to acoustic streaming and agitation of the final irrigant. Compared to the finder system the endoactivator was more efficient though not statistically significant.

Boutsiokis et al in 2010 [15] analyzed the effect of needle insertion depth on irrigant flow in the root canal using a unsteady computational fluid dynamics model and observed that needle insertion depth was found to affect the extent of irrigant replacement, shear stress on the canal wall and the pressure at the apical foramen for both the canal types. Positioning the needle closer to the working length improved irrigant displacement at the apical part of the canal, but also increased the mean pressure at the apical foramen indicating a increased risk of extrusion. Variations in needle position and the canal taper had to be taken into account to decide the ideal needle position for each case.

A scanning electron microscopic study on the effect of various root canal irrigants on removal of smear layer and debris was done by **Balaji T.S. et al in 2010.**[8] He observed that 5% ethylene diamine, 17% EDTA mixture was as efficient as 17%EDTA and 4%sodium hypochlorite when used alternatively as irrigants during root canal preparation and when used could be less time consuming than use of alternative solution.

Caron et al in 2010 [19] examined the effect of different final irrigation regimens and methods of activation on smear layer removal in curved canals after root canal instrumentation and concluded that root canal cleanliness benefits from irrigant solution activation especially sonic and manual dynamic activation in comparison with no activation during the final irrigation regimen. They also observed that a tapered tip which closely resembles the final canal preparation to be most effective.

The effect of aquatine endodontic cleanser on smear layer removal in root canals were evaluated by **Garcia F. et al in 2010** [34] in an ex-vivo study and observed that aquatine EC had similar effectiveness as 6% sodium hypochlorite when used with a rinse of EDTA to clean the root canals of debris and smear layer following contamination with Enterococcus Faecalis. They also pointed out that aquatine EC may be superior to sodium hypochlorite in terms of biocompatibility, and could provide a safer alternative to sodium hypochlorite for removal of biofilms of bacteria in root canals.

Hariharan VS, Nandalal et al in 2010 [39] in their study evaluated the efficacy of root canal irrigants on smear layer removal in primary root canals using scanning electron microscope. They compared saline, 5.25% sodium hypochlorite, 10% EDTA + 5.25% sodium hypochlorite, 6% Citric acid and 2% Chlorhexidine gluconate and observed that 10% EDTA and 5.25% sodium hypochlorite caused unwanted damage to the dentinal tubules though it removed the smear layer. 5.25% sodium hypochlorite, saline and 2%Chlorhexidine do not have the potential to remove the smear layer, where sodium hypochlorite appears comparatively better. They found that 6%Citric acid to be very efficient at smear layer removal and recommended that saline can be used during instrumentation; final irrigation should be done with 6% citric acid followed by 2% chlorhexidine to potentiate antimicrobial action and substantivity, in primary teeth during endodontic therapy.

Jiang et al in 2010 [44] evaluated the removal of dentin debris from the root canal by sonic or ultrasonic activation of the irrigant and the physical mechanisms of sonic activation by visualizing the oscillations of the sonic tip both inside and outside the confinement of the root canal. They observed that the activation of the irrigant resulted in significantly more dentin debris removal. Ultrasonic activation was significantly more efficient than sonic activation. They also found that the oscillation amplitude of the sonically driven tips is 1.2+/- 0.1mm resulting in much wall contact and no cavitation of the irrigant.

On the review of root canal irrigants by **Kandaswamy .D and Venkatesh Babu .N et al in 2010** [45] they observed and concluded that during root canal instrumentation the canals should be copiously irrigated with sodium hypochlorite. Once the shaping procedure is completed they should be rinsed with EDTA or citric acid for a minimum of 1 minute with a 5 to 10ml of the chelator agent. After the smear layer removal procedure a final rinse with an antiseptic solution appears beneficial and chlorhexidine appears to be promising in this regard as it has the property of substantivity.

A new irrigant regimen has been advocated with the introduction of MTAD as an endodontic irrigant. The recently revised protocol for clinical use of MTAD advises an initial irrigation for 20 minutes with 1.3% sodium hypochlorite followed by a 5 minute final rinse with MTAD. They also observed that use of MTAD had a negative effect on the bonding ability of both resin and calcium hydroxide based sealers due to precipitate formation. They also suggested the need for development of a single irrigant that has tissue dissolving ability, smear layer removal property and antimicrobial efficacy.

Mello et al in 2010 [57] studied the influence of the final rinse technique on the ability of 17% EDTA (Ethylene Diamine Tetra acetic Acid) on the removal of smear layer in an in-vitro setting and concluded that a continuous rinse of 5ml of 17% EDTA for 3 minutes can effectively remove smear layer from all areas of root canals. They recommended the use of the decalcifying agent as a final rinse in effectively removing the smear layer. They also noted that the volume of EDTA and the length of time used in this study did not cause significant undesired alteration in the dentinal structure.

Paragliola et al in 2010 [67] examined the effect of different agitation protocols in the penetration of an endodontic irrigant into dentinal tubules and concluded that the use of ultrasonic agitation increases the effectiveness of the final rinse procedure in the apical third of the root canal walls.

The efficacy of subsonic agitation of sodium hypochlorite in reducing the bacterial load in the root canal was evaluated by **Pasgualini et al in 2010**.[68] They concluded that sodium hypochlorite subsonic agitation for 30 seconds appeared to be slightly more effective in reducing the bacterial load in the root canal compared with sodium hypochlorite irrigation alone.

Rodig et al in 2010 [75] evaluated the cleaning efficacy of different irrigant agitation techniques on debris and smear layer removal in curved root canals and concluded that sodium hypochlorite and EDTA did not enhance debris removal but resulted in significantly more effective smear layer removal at coronal levels. Root canal cleanliness was better at coronal than in the apical root canal region.

Rodig et al in 2010 [74] evaluated the debris removal from simulated root canal irregularities using vibringe system with syringe and passive ultrasonic irrigation and concluded that passive ultrasonic irrigation was more effective than vibringe system or syringe irrigation in debris removal. The sonic device demonstrated significantly better results than syringe irrigation in the apical root canal third. They also found that none of the tested devices were able to completely remove the debris from artificial extensions in straight root canals.

Santos M C et al in 2010 [82] did a scanning electron microscopic analysis of smear layer removal by doxycycline and concluded that when used together EDTA and sodium hypochlorite solutions were effective in removing the smear layer. Doxycycline 100mg/ml when used alone was effective in the cervical and middle thirds but less effective in the apical third.

On an in-vitro study of three dimensional numeric simulation of irrigant flow with different needle designs **Shen et al in 2010** [91] concluded that needle tip design influences flow pattern, flow velocity and apical wall pressure which are important parameters for effectiveness and safety of irrigation. They emphasized the need for a continuous research on needle tip design. This study was based on a computational fluid dynamics model.

Uroz Torres et al in 2010 [104] evaluated the effectiveness of the endoactivator system in removing the smear layer after rotary root canal instrumentation with and without the use of a final flush of a 17% EDTA solution, in the coronal, middle and apical thirds. They concluded that the endoactivator system was not as effective as conventional max-I probe irrigation with sodium hypochlorite and EDTA in smear layer removal.

Vivian R.R. et al in 2010 [110] analyzed the Rinse Endo and conventional irrigation system for debris removal using scanning electron microscope.The rinse endo-system compared to static and conventional irrigation was believed to be more effective for cleaning the canal walls due to their greater ability of penetration. However there was no difference between the Rinse Endo system and conventional irrigation in the cleaning ability of root canals. No differences were found in the canal thirds regarding the debris removal.

Blank-Gonclavos et al in 2011 [13] evaluated the effectiveness of different irrigant agitation techniques on smear layer removal in curved root canals. They observed that the activation systems removed significantly more smear layer than conventional irrigation in the apical third of the root canals.

Dadresanfar B, Khalilak Z, Delvarani.A, et al in 2011 [22] studied the effect of ultrasonication with EDTA (Ethylene Diamine Tetra Acetic acid) or MTAD (Mixture of Tetracycline isomer Acid and Detergent) on smear layer and found that when used in accordance with the manufactures protocol MTAD appears to induce less dentinal erosion with proper removal of the smear layer and debris in wide canals. They also found that passive ultrasonic agitation of EDTA also increases dentin erosion.

The effect of QMix an experimental antibacterial root canal irrigant on removal of canal wall smear layer and debris was evaluated by **Dai et al in 2011**[23] in an invitro setting. They found that the apical third of the canal is the most difficult to clean which was consistent with other studies. They concluded that both the versions of QMix were as effective as EDTA in smear layer removal from the canal walls. They also found that similar to Biopure MTAD and EDTA these QMix versions were ineffective in cleansing debris completely from the root canal spaces when the corresponding irrigant was delivered via the insertion of a side vent needle within 1mm of the apical seal. They also suggested future evaluation of debris removal in a closed canal system in conjunction with sonic or ultrasonic activation or devices using an apical negative pressure approach. QMix used in this study was a experimental mixture of bis-biguanide (antimicrobial agent) a polyamino carboxylic acid (calcium chelating agent) and a surfactant. Two versions of QMix with a pH of 8.0 and 7.5 were used in this study.

The wetability of endodontic sealers in contact with dentin treated with 5.25% sodium hypochlorite and 2%chlorhexidine in presence or absence of smear layer was evaluated by **De Assis et al in 2011** [25] and found that smear layer removal and final flush with chlorhexidine favour the wettability of AH plus and Real Seal SE sealers. They also noted that water wettability alone is not a good parameter for evaluating the sealers adhesiveness and for this reason the sealers hydrophobic characteristic should be taken into account. sodium hypochlorite did not favour spread of AH plus.

Peeters and Suardita in 2011[70] compared the efficacy of LASER driven irrigation in removing smear layer and debriding the apical region of the root canal (the root tip) with that of ultrasonic irrigation, in a invitro setting and found that the use of a LASER with a plain fiber tip which produces cavitation in the irrigant has potential as an improved alternative method for removal of smear layer from the apical region of a straight canal. The irrigant used in this study was 17%EDTA.

Saber and Hashem in 2011 [79] compared the smear layer removal after final irrigant activation with apical negative pressure, minimal dynamic agitation and passive ultrasonic irrigation. They observed that irrigant activation with apical negative pressure and manual dynamic agitation resulted in better removal of the smear layer compared to passive ultrasonic irrigation or passive irrigation.

Tikku et al in 2011 [99] reviewed the role of Titanium tetra Fluoride (TiF_4) as a root canal irrigant in endodontics and observed that there has been an enormous amount of research and debate on the advantages and disadvantages of removing smear layer before obturation and a mid pathway of modifying the smear layer in a way that it becomes completely resistant to dissolution and disintegration has been conceptualized, which also blocks the dentinal tubules permanently. Such a promising biochemical and biomechanical change has been observed when treated with titanium tetra fluoride irrespective of the presence or absence of smear layer The smeared surface showed a thicker coating (1-5µm). It has also been shown that the interaction of titanium tetra fluoride and smear layer produces a stable, acid resistant structure indicating its potential role in reducing microleakage and improving apical seal of the root canal system.

Vera et al in 2011 [105] evaluated whether the use of a patency file is related to the presence of a radio-opaque irrigating solution in the apical third of human root canals after using passive ultrasonic activation in-vivo and concluded that maintaining apical patency and then using passive ultrasonic irrigation improves the delivery of irrigants into the apical third of the root canals.

Wadhwani K.K. et al in 2011 [111] compared the smear layer removal using two rotary systems with EDTA in different states using a scanning electron microscope. They used M-two and protaper rotary instruments and EDTA (Ethylene Diamine Tetra Acetic acid) as an irrigant solution and gel. They observed no statistically significant difference was found between the EDTA solution and gel, and that both the NiTi instruments produced similar dentin surface on the root canal wall for all parameters considered.

ARMAMENTARIUM

Collection of teeth

1. Normal saline (Nirlife Health Care, Nirma Products, India)
2. 2% Thymol solution(Alpha Chemicals, Maharastra, India)
3. Vented glass bottles
4. Tissue forceps

Selection & Preparation of samples

1. RadioVisuoGraphy Satelec RVG (Satelec X- Mind Ac / Dc radiography unit, Italy)
2. Diamond disc
3. Polyvinyl siloxane impression material (Panasil putty soft, Kettenbach GmbH & Co, Germany)
4. Small transparent plastic containers for sample placement
5. Magnifying Lens with Illumination
6. Modeling wax (Hiflex –Prevest Denpro Limited, Jammu, India)
7. Wax carvers
8. Spirit lamp
9. Indelible marker

Root canal preparation

1. Size 8,10,15 K file of 21mm length (Dentsply, Maillefer, Ballaigues, Switzerland)
2. 5ml syringe with leur-lock needle (Dispovan, Hindustan Syringes and Medical Devices Ltd, Faridabad, India)

3. 28 gauge side-vent needle (Dentsply, Tulsa dental, Tennessee, USA)
4. 5ml syringe unilock (Hindustan Syringes and Medical Devices Ltd, Faridabad, India)
5. Endo block (Dentsply Maillefer, Ballaigues, Switzerland)
6. Endomotor (X-smart with 1:16 reduction hand piece- Dentsply Maillefer, Ballaigues, Switzerland)
7. Protaper rotary file system (21mm- S1,S2,F1,F2,F3) –(Dentsply Maillefer, Ballaigues, Switzerland)
8. Gutta percha points F3 (Dentsply Maillefer, Ballaigues, Switzerland)
9. Ultrasonic unit- (EMS)
10. Endosonic tips stainless steel with adaptor (EMS)
11. Sonic activation – Endoactivator (Dentsply Maillefer, Ballaigues, Switzerland)
12. Fibre tips - size 25 for Endoactivator (Dentsply Maillefer, Ballaigues, Switzerland)

Irrigating solutions

1. Normal saline (Nirlife Health Care, Nirma Products, India)
2. 5% Sodium Hypochlorite solution (Nice chemicals Pvt Ltd, India)
3. 1.3% Sodium Hypochlorite solution (Nice chemicals Pvt Ltd, India)
4. 17% EDTA solution (pulpdent corporation, USA)
5. Sterile Distilled water (Ives drugs, Pvt Ltd, India)
6. Biopure MTAD (Dentsply, Tulsa dental specialties, Tennessee, USA)
7. Electro chemically activated water – Sterilox (Sterilox Technologies international limited, Stafford, U.K.)

Sectioning of samples

1. Diamond disc
2. 0.5 inch Stainless Steel bibeveled chisel
3. Stainless steel mallet

Preparation for SEM analysis

1. Ascending concentrations of Isopropyl alcohol (S.V. Drugs and chemicals, Faridabad, India)
2. Sterile self sealing pouches (AK Product; West Bengal; India)
3. U-V light chamber (Apex Industrial Electronics, Haryana, India)

Scanning electron microscopic analysis

1. Scanning Electron Microscope (S-3400N; HITACHI, Japan)
2. Gold Sputter coating machine (E-1010; HITACHI, Japan)
3. Carbon tape (Royal tapes Pvt Ltd., Chennai, India)
4. Storage media

Image analysis

1. Sony VIAO computer
2. Image analysis software (EDS software).

MATERIALS AND METHODS

1. Collection of teeth:

One hundred and sixty extracted human permanent, single rooted maxillary incisors, maxillary canines and mandibular first premolars were collected and stored in isotonic saline solution for a maximum of 72 hours. Protocols for infection control as per OSHA and CDC guideline regulations in collection, storing, sterilization and handling were followed.

2. Selection of samples:

Teeth devoid of caries, restorations endodontic treatments were separated. They were then observed for cracks and such teeth were excluded. Teeth with mature and intact root apices were selected for the purpose of the study. The selected teeth were then analyzed using digital radiography to ensure that they had a single patent canal and the root lengths were a minimum of 15mm (measured from the tip of the root to the cemento-enamel junction).The selected teeth were then stored in normal saline solution at 4°C until use. A total of one hundred and fifty teeth were selected for the purpose of the study.

3. Standardization of samples

The working length was determined by passively placing a size 8K file (Dentsply Maillefer, Ballaigues, Switzerland) into the canal until the tip was visualized at the apical foramen using a magnifying loupe and was adjusted to the apical foramen. Then the actual canal length was measured and working length was calculated by subtracting 0.5mm from this measurement.

4. Preparation of the Sample

The selected teeth were then decoronated and standardized to a length of 15mm (working length) by sectioning with a diamond disc under water spray. The samples were rinsed with distilled water and stored in normal saline at 4°C.

The teeth were then dried and modeling wax was applied at the apical foramen. They were then placed in a transparent small plastic container into which a soft poly-vinyl siloxane impression material had been placed and excess material trimmed off. The aim was to prevent the irrigants from extruding the apex in order to simulate in-vivo conditions. The samples were then randomly divided in to six experimental groups (n=12). Comprising of twelve teeth each and two control groups (n=5).

5. Root Canal Preparation Technique

The instrumentation was initiated with hand files (Dentsply, Maillefer, Ballaigues, Switzerland) upto size 20 followed by protaper rotary files from size S1-F3.The root canals of the samples were prepared using protaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) with X-smart endomotor (Dentsply Maillefer, Ballaigues, Switzerland) as per the manufacturer instructions. The irrigant was delivered using a 28- gauge side vent pro-rinse needle (Dentsply, Tulsa Dental) at the working length. 1ml of the irrigant was used for canal irrigation after using each instrument and before proceeding to the next.

6. **Final Rinse of Samples**

Subsequent to the canal preparation the samples were irrigated with a final rinse of 5ml of the irrigant as per the respective group. The delivery of MTAD as final rinse was done as per the manufacturer protocol for use as final rinse (Dentsply, Tulsa dental). For all other irrigants used as final rinses a total of 5ml of the irrigant was delivered using a 28- gauge side vent pro-rinse needle (Dentsply, Tulsa dental) for duration of three minutes.

During the first minute delivery of the irrigant, the needle was withdrawn to 5mm inserted back to working length followed by rotation of the needle by 180° three times alternatively.

During the second minute a F3 size gutta percha cone (Dentsply Maillefer, Ballaigues, Switzerland) was inserted to working length and withdrawn three times (Manual Dynamic Activation). This was done to improve the irrigant delivery and replacement to the apical third of the canal space.

The experimental groups (Groups IV –VIII) were further subdivided into subgroups which were then subjected to sonic and passive ultrasonic activation. Sonic activation was done using an endoactivator unit (Dentsply Maillefer, Ballaigues, Switzerland) with the tip at 2mm short of working length and activated for twenty seconds three times intermittently. Passive ultrasonic activation was done with an Endosonic EMS unit with the tip at 2mm short of working length and activated intermittently for twenty seconds.

After the completion of three minutes a post-final rinse irrigation of 10ml of distilled water was done to flush out the remaining final rinse irrigant from within the canal.

7. Preparation of samples for SEM analysis

The sectioned teeth after removal from the poly-vinyl siloxane base were covered with cotton wool at the orifice and subsequently grooved longitudinally on the external surface in a bucco-lingual plane with a diamond bur with sufficient care not to accidentally penetrate the root canals. The teeth were then split longitudinally in a bucco-lingual plane dividing them into two halves using a mallet and a chisel. For each tooth the half containing the most visible part of the apex was selected, stored and coded.

The teeth were then placed in a 10% neutral buffered formalin solution at 18°C for 24 hours. They were then post fixed in Osmium Tetroxide (1%w/v) for two hours before being dehydrated in graded solutions of Isopropyl alcohol (S.V. Drugs and chemicals, Faridabad, India).

The teeth were then placed in a filter paper for 24 hours, separation markings of 5mm made for apical, middle and coronal thirds and irradiated with UV light in a UV light sterilization chamber and stored in sterile pouches. Each group was processed and stored separately.

8. SEM Examination:

The coded samples of each group were mounted on to aluminium stubs with carbon tape (Royal tapes Pvt Ltd., Chennai, India) with the entire root canal visible and facing upwards. Each of the specimens was coated with a 20-30nm thin layer of gold in a gold sputter coating machine (HITACHI, Japan). The samples were then examined using a scanning electron microscope (HITACHI, Japan). The SEM photo micrographs were obtained at X2000 magnification using digital image analysis software and stored appropriately for subsequent analysis. The most representative micrographs were taken for each millimeter of the specimen. Fine micrographs were recorded for apical, middle and coronal thirds respectively.

9. Analysis of photomicrographs

The photomicrographs were analyzed after coding based on the representative groups in a blind manner by two independent investigators for the presence of smear layer, debris and erosion in the coronal, middle and apical thirds of each specimen.

The **smear layer** was analyzed using the following criteria**(Caron et al 2010).**

Score 1: No smear layer and dentinal tubules open.

Score 2: Small amounts of scattered smear layers and dentinal tubules open.

Score 3: Thin smear layer and dentinal tubules partly open. (Crescent shaped)

Score 4: Thick smear layer with partial covering of dentinal tubules.

Score 5: Total covering with thick smear layer.

The presence of **debris** was analyzed using the following criteria **(Dadresenfar et al in 2011)**

Score 1: Clean canal wall, few debris particles.

Score 2: Few conglomerations.

Score 3: Many conglomerations less than 50% of canal wall.

Score 4: More than 50% of canal wall with conglomerations.

Score 5: Complete or near complete covering of canal wall by debris.

The presence of **erosion** was analyzed by using the following criteria **(Torabinejad et al in 2003)**

Score 1: No erosion (All tubules normal in appearances)

Score 2: Moderate erosion (Peritubular dentin eroded)

Score 3: Severe erosion (Intertubular dentin destroyed and tubules connected to each other)

10. Tabulation of result and statistical analysis

The results which were scored by the independent operators were compared and tabulated for their respective score values of smear layer, debris and erosion in the apical, middle and coronal thirds. The results were then statistically analyzed.

TABLE – 1 IRRIGATION GROUPING

Groups (n=5-12)	Irrigating Solution	Final Rinse
I - Negative Control (n=5)	Normal Saline	Normal Saline
II – Positive Control (n=5)	5% NaOCl	17% EDTA
III	STERILOX	17% EDTA
IV	STERILOX	Biopure MTAD
V	1.3% NaOCl	STERILOX
VI	5% NaOCl	STERILOX
VII	1.3% NaOCl	Biopure MTAD
VIII	5% NaOCl	Biopure MTAD

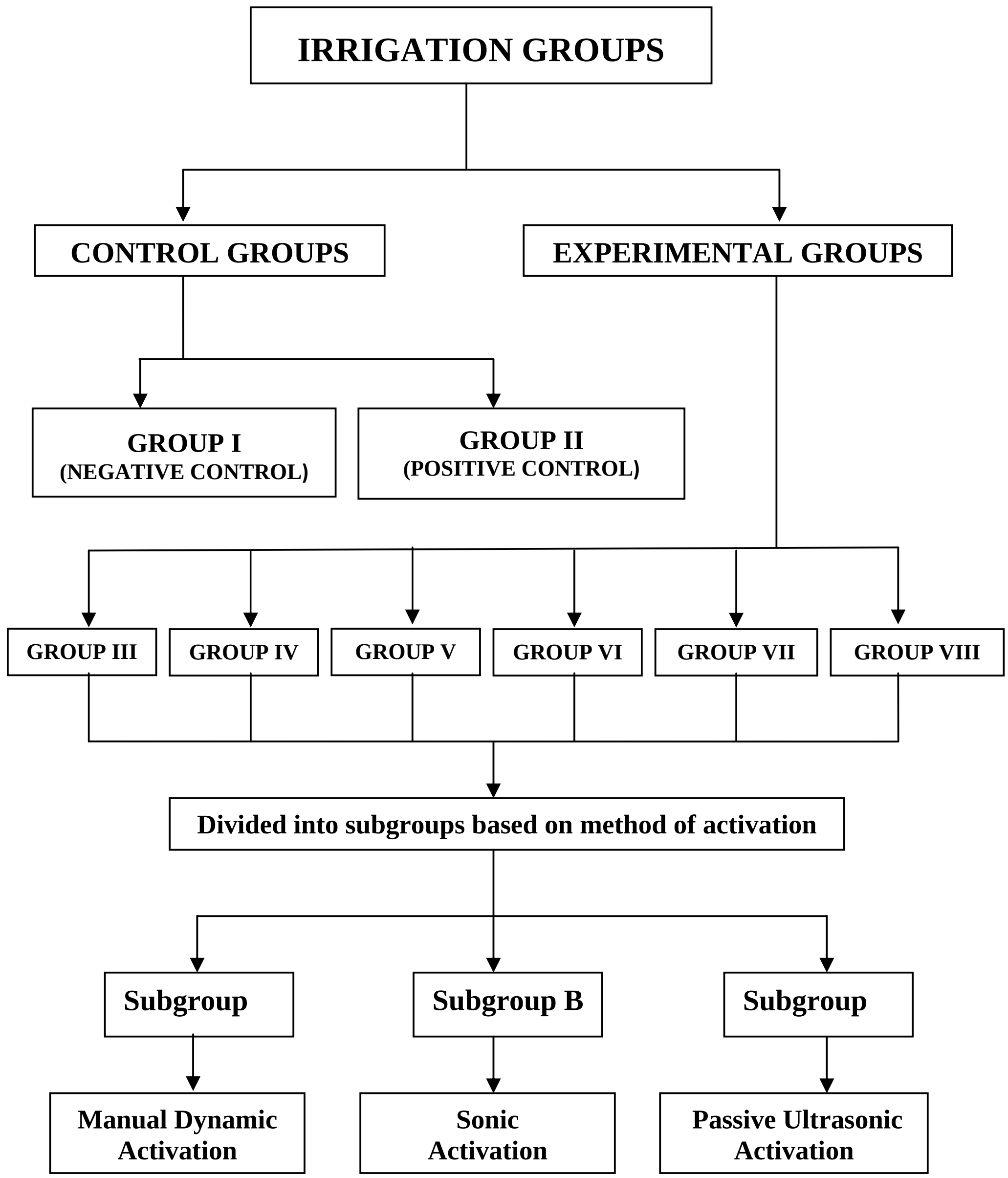
IRRIGATION GROUPS
CONTROL GROUPS
EXPERIMENTAL GROUPS
GROUP I
(NEGATIVE CONTROL)
GROUP II
(POSITIVE CONTROL)
GROUP III
GROUP IV
GROUP V
GROUP VI
GROUP VII
GROUP VIII
Divided into subgroups based on method of activation
Subgroup
Subgroup B
Subgroup
Manual Dynamic Activation
Sonic Activation
Passive Ultrasonic Activation

TABLE-2 AVERAGE SMEAR LAYER SCORES

GROUPS	APICAL	MIDDLE	CORONAL
I	5	5	5
II	1.7	1	1
III	2.7	1	1
IV	2.7	1.63	1.3
V	4	3.4	2.4
VI	3.8	3.3	2.7
VII	2.7	1	1
VIII	1.6	1	1
MEAN	**3.0250**	**2.1663**	**1.9250**
SD	**1.1683**	**1.5384**	**1.4190**

CHART: 1

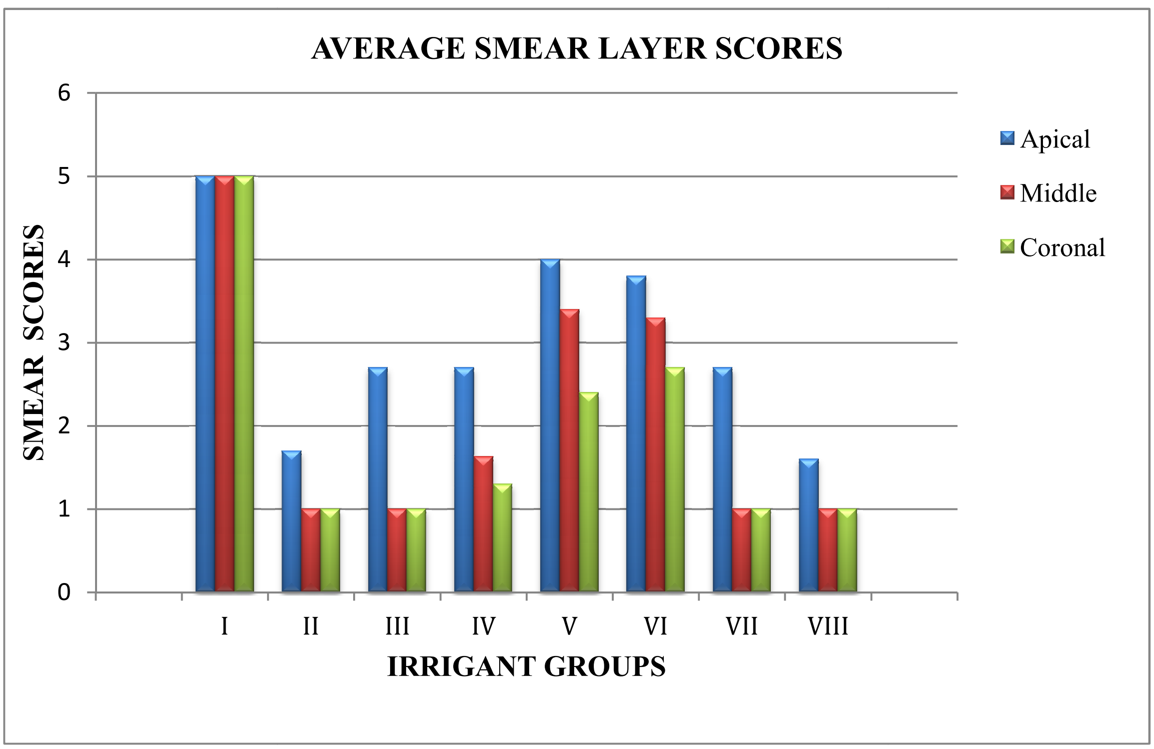

CHART: 2 DISTRIBUTION OF SMEAR SCORES AT APICAL, MIDDLE AND CORONAL THIRDS

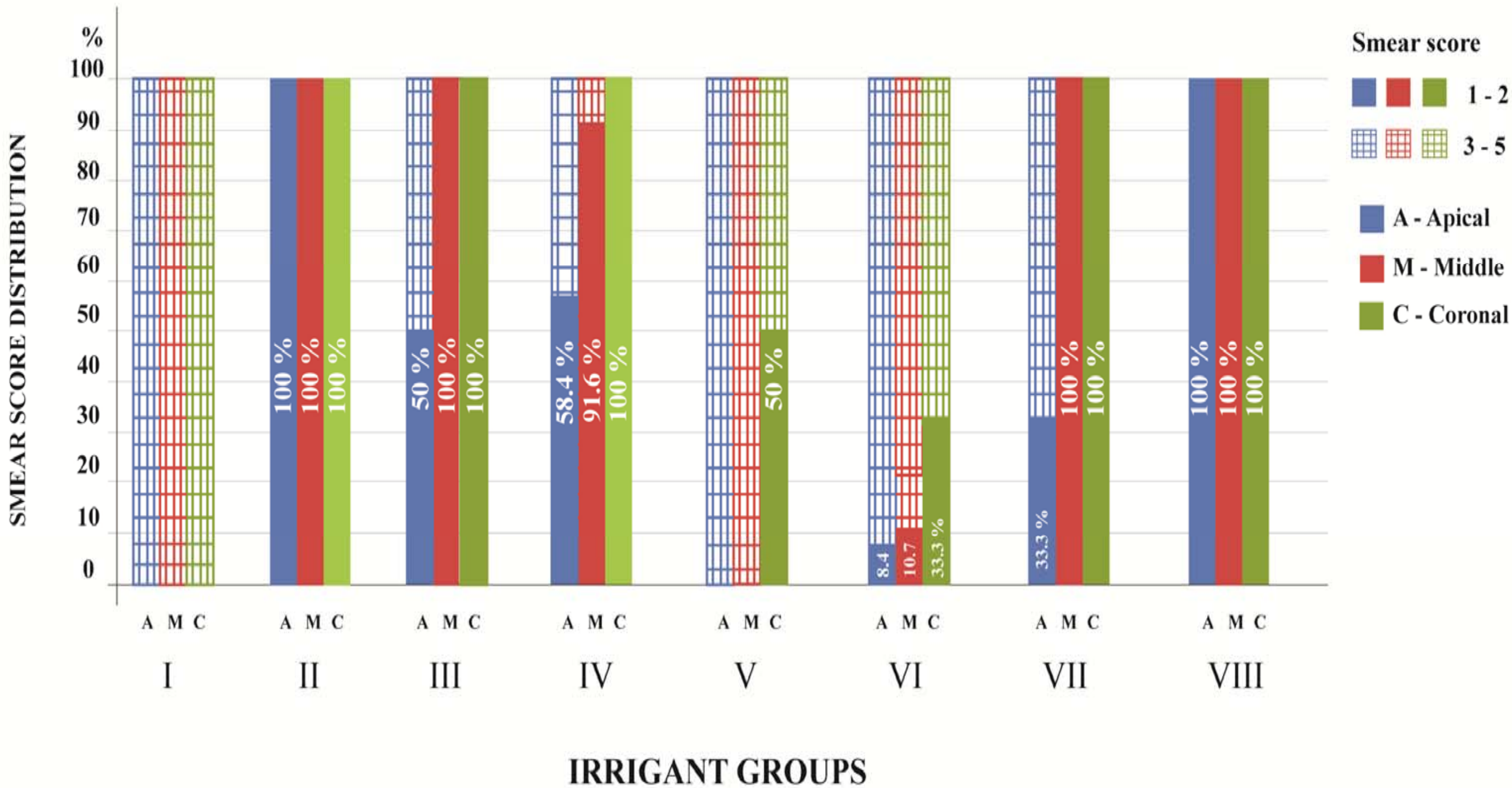

TABLE -3 STATISTICAL ANALYSIS OF SMEAR SCORES USING STUDENT'S (t) TEST AND MANN-WHITNEY U TEST

Groups compared	t value	p value	Statistical significance	z value	p value	Statistical significance
I & II	16.143	0.001	**A significant difference**	2.121	0.034	**A significant difference**
II & III	0.544	0.615	No significant difference	0.796	0.258	No significant difference
IV & VII	0.439	0.684	No significant difference	0.899	0.369	No significant difference
IV & VIII	1.447	0.221	No significant difference	1.550	0.121	No significant difference
V & VII	4.841	0.038	**A significant difference**	1.550	0.121	No significant difference
VI & VIII	5.502	0.005	**A significant difference**	1.993	0.046	**A significant difference**

Interpretation:

1. **5% (0.05)** level of significance considered in all the comparisons
2. If **'p' value is less than 0.05** we conclude that there is **a significant difference** between the two groups
   If **'p' value is more than 0.05** we conclude that there is **no significant difference** between the two groups
3. **Student's- t test (t- value)** is used to test the difference between two groups regarding **Mean**
4. **Mann-Whitney U test (z- value)** is used to test the difference between two groups regarding **Ranks**

TABLE-4 AVERAGE DEBRIS SCORES

GROUPS	APICAL	MIDDLE	CORONAL
I	4.6	4.6	4.2
II	1.3	1	1
III	2.8	1.3	1
IV	1.8	1.3	1.3
V	2	2.6	1.6
VI	1.5	1.3	1.6
VII	1.4	1	1
VIII	1.2	1	1
MEAN	**2.0750**	**1.7625**	**1.5875**
SD	**1.1424**	**1.2614**	**1.0882**

CHART: 3

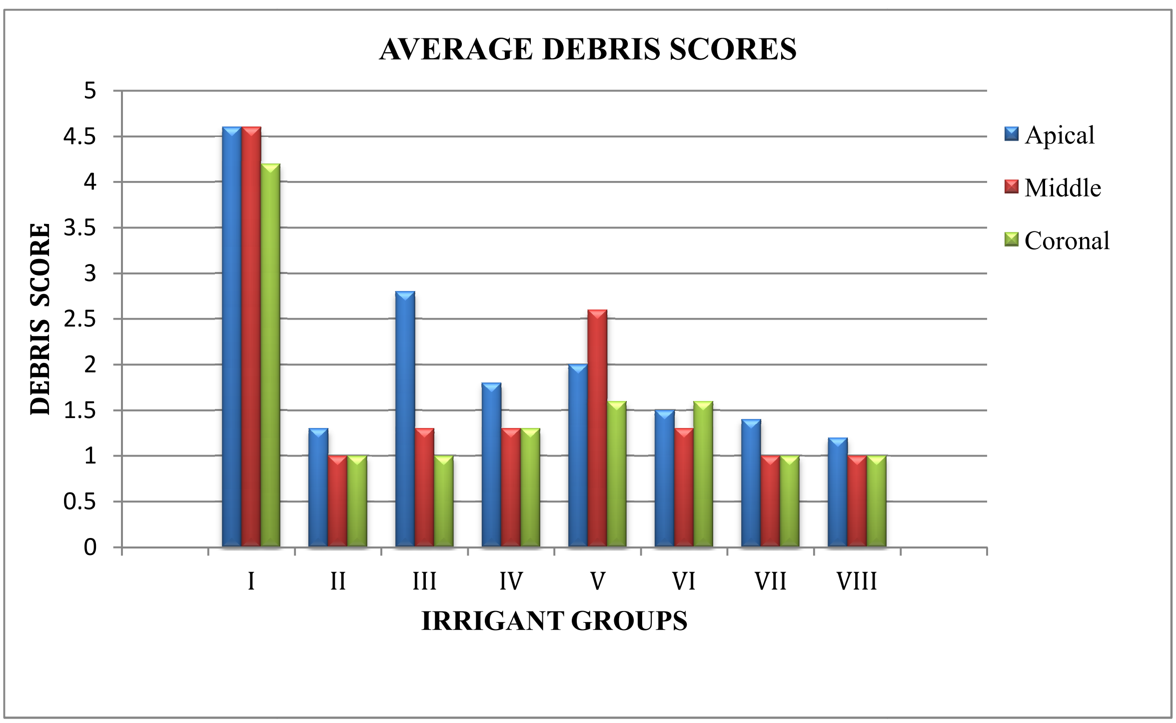

CHART: 4 DISTRIBUTION OF DEBRIS SCORES AT APICAL, MIDDLE AND CORONAL THIRDS

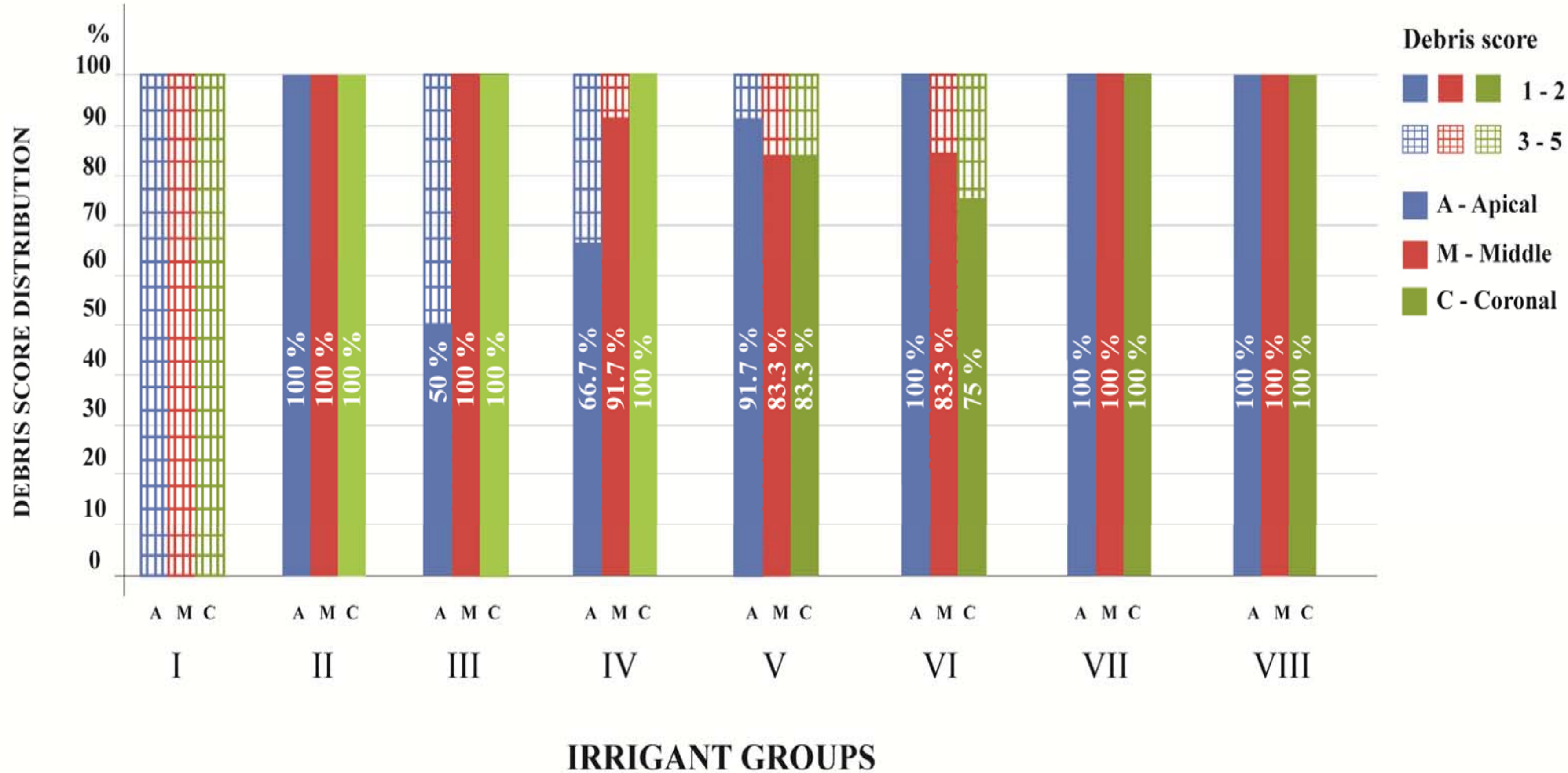

TABLE -5 STATISTICAL ANALYSIS OF DEBRIS SCORES USING STUDENT'S (t) TEST AND MANN-WHITNEY U TEST

Groups compared	t value	p value	Statistical significance	z value	p value	Statistical significance
I & II	20.201	0.001	**A significant difference**	2.023	0.043	**A significant difference**
II & III	1.710	0.162	No significant difference	0.943	0.346	No significant difference
IV & VII	1.562	0.193	No significant difference	1.124	0.261	No significant difference
IV & VIII	2.228	0.090	No significant difference	2.023	0.043	**A significant difference**
V & VII	2.919	0.043	**A significant difference**	1.993	0.046	**A significant difference**
VI & VIII	3.213	0.057	No significant difference	1.993	0.046	**A significant difference**

Interpretation:

1. **5% (0.05)** level of significance considered in all the comparisons
2. If **'p' value is less than 0.05** we conclude that there is **a significant difference** between the two groups
   If **'p' value is more than 0.05** we conclude that there is **no significant difference** between the two groups
3. **Students-t test (t- value)** is used to test the difference between two groups regarding **Mean**
4. **Mann-Whitney U test (z- value)** is used to test the difference between two groups regarding **Ranks**

TABLE-6 AVERAGE EROSION SCORES

GROUPS	APICAL	MIDDLE	CORONAL
II	2.8	2.4	2.6
III	1.8	1.6	1.8
IV	1.8	1.9	1.9
VII	1.3	1.8	1.8
VIII	2.8	2	1.5
MEAN	**2.1**	**1.94**	**1.92**
SD	**0.6708**	**0.2966**	**0.4087**

CHART: 5

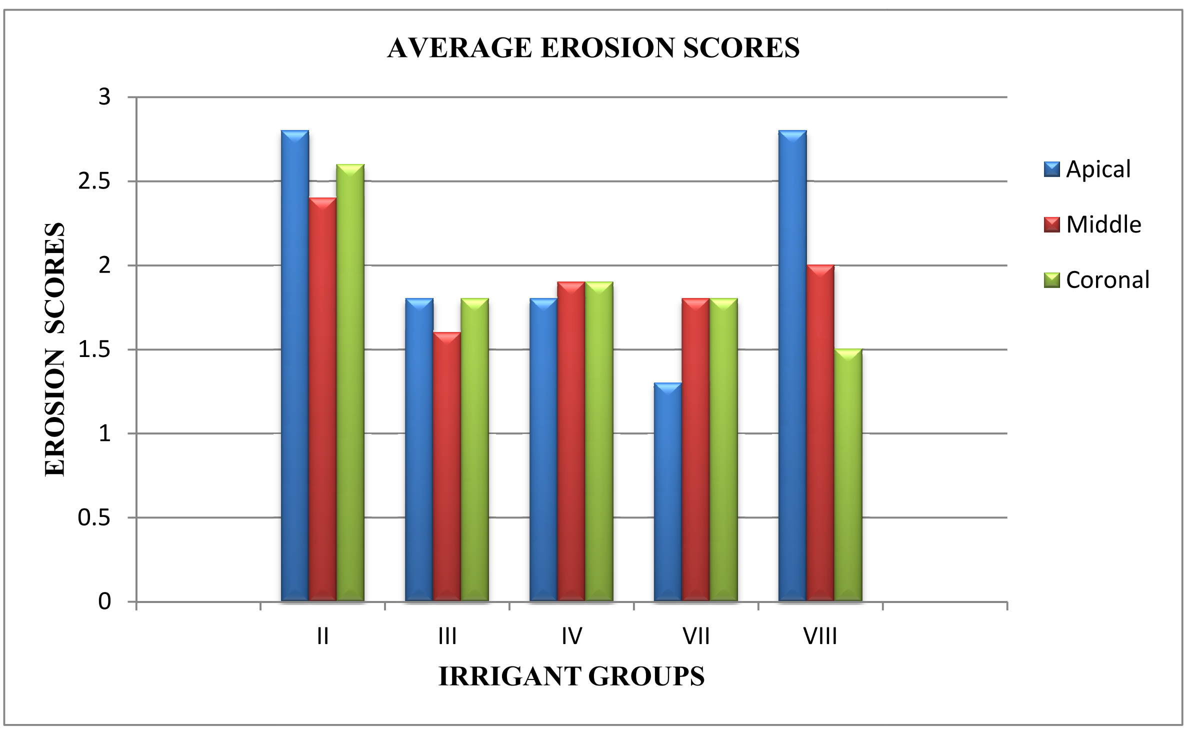

CHART: 6 DISTRIBUTION OF EROSION SCORES AT APICAL, MIDDLE AND CORONAL THIRDS

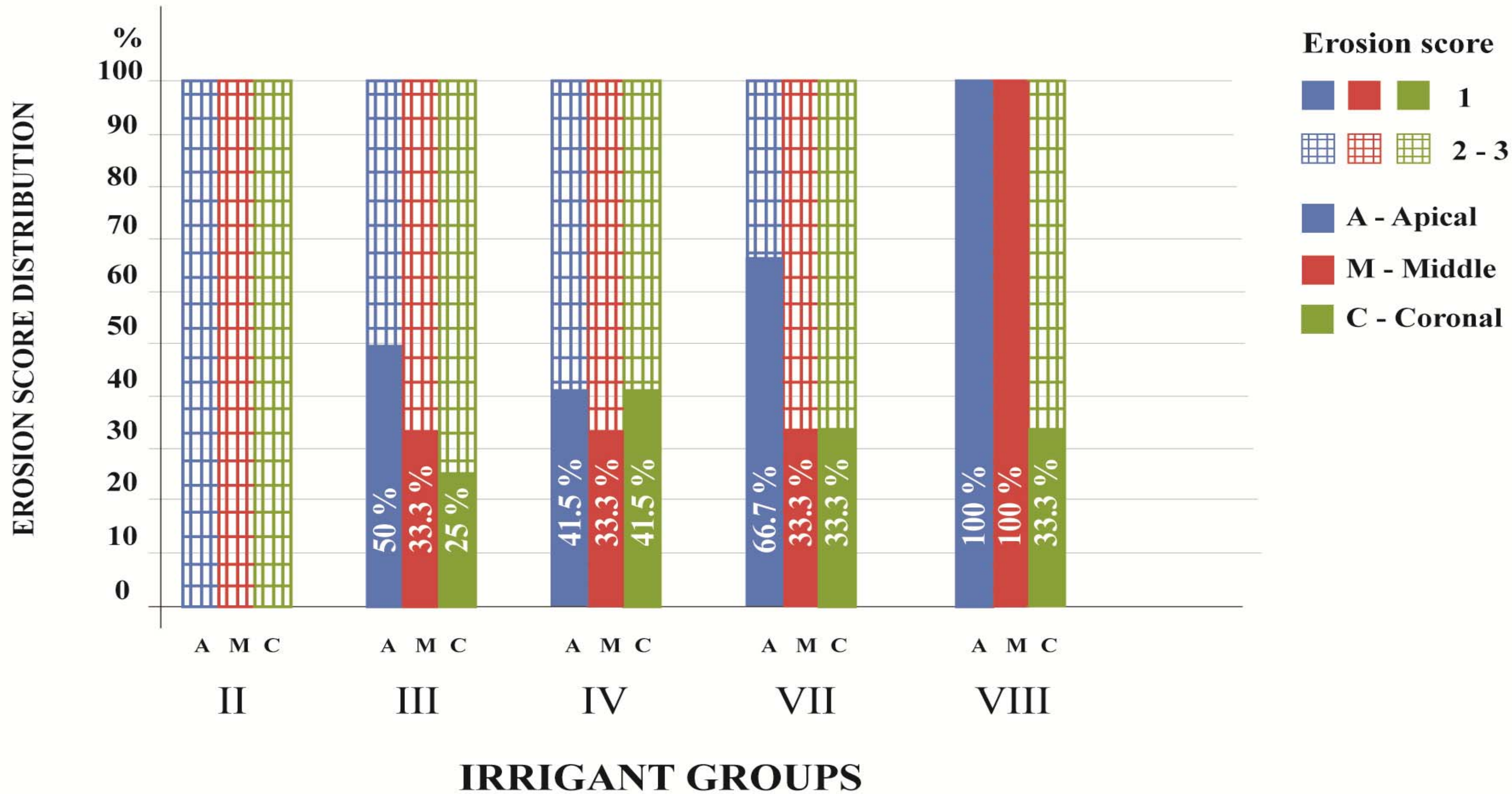

TABLE-7 MEAN SCORERS

Mean values	Apical	Middle	Coronal
Smear	3.0250	2.1663	1.9250
Debris	2.0750	1.7625	1.5875
Erosion	2.1	1.94	1.92

CHART: 7

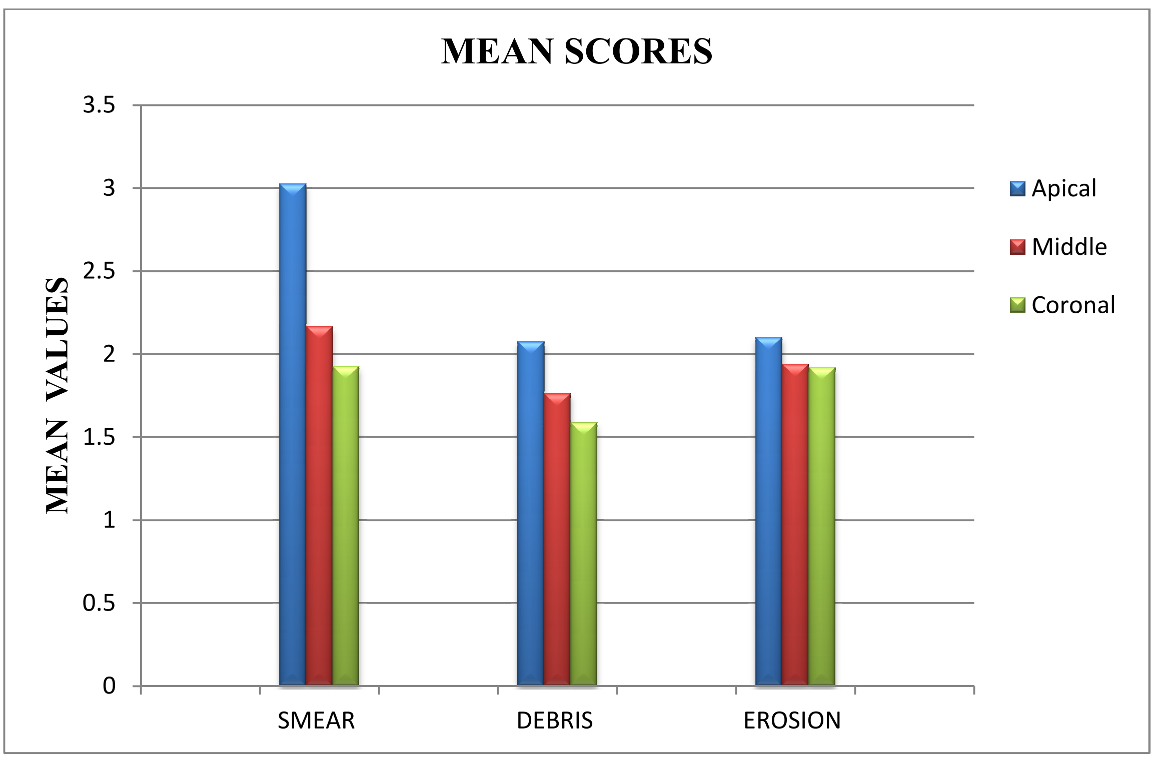

TABLE-8 AVERAGE SMEAR SCORES FOR SUBGROUPS

Irrigant groups		Manual Dynamic activation	Sonic activation	Passive ultrasonic activation	Mean	SD
III	**Apical**	2.8	2.3	2.5	**2.5333**	**0.2517**
	Middle	1	1	1	**1**	**0**
	Coronal	1	1	1	**1**	**0**
IV	**Apical**	2.8	3	2.6	**2.8**	**0.2**
	Middle	1.3	1.5	2.4	**1.7333**	**0.5859**
	Coronal	1	1.8	1.3	**1.3667**	**0.4041**
V	**Apical**	4	3.9	3.2	**3.7**	**0.4359**
	Middle	4.2	3.5	2.8	**3.5**	**0.7**
	Coronal	3	2.6	1.3	**2.3**	**0.8889**
VI	**Apical**	4.7	3.8	2.8	**3.7667**	**0.9504**
	Middle	4	2.8	3.3	**3.3667**	**0.6028**
	Coronal	3.8	2.3	2.2	**2.7667**	**0.8963**
VII	**Apical**	3.8	1.3	2.4	**2.5**	**1.2530**
	Middle	1	1	1	**1**	**0**
	Coronal	1	1	1	**1**	**0**
VIII	**Apical**	1.3	1.3	1	**1.2**	**0.1732**
	Middle	1	1	1	**1**	**0**
	Coronal	1	1	1	**1**	**0**

TABLE-9 AVERAGE DEBRIS SCORES FOR SUBGROUPS

Irrigant groups		Manual dynamic activation	Sonic activation	Passive ultrasonic activation	Mean	SD
III	Apical	2.8	2.4	2.3	**2.5**	**0.2646**
	Middle	1.3	1.3	1.3	**1.3**	**0**
	Coronal	1	1	1	**1**	**0**
IV	Apical	1.8	2	1.8	**1.8667**	**0.1155**
	Middle	1	1.5	1.5	**1.3333**	**0.2887**
	Coronal	1	1.4	1.5	**1.3**	**0.2646**
V	Apical	2	2.3	1.8	**2.0333**	**0.2517**
	Middle	4	2	2	**2.6667**	**1.1547**
	Coronal	2	1.5	1.5	**1.6667**	**0.2887**
VI	Apical	1.5	2	1.25	**1.5833**	**0.3819**
	Middle	1	1.5	1.5	**1.3333**	**0.2887**
	Coronal	1	1.8	2	**1.6**	**0.5292**
VII	Apical	2	1.25	1	**1.4167**	**0.5204**
	Middle	1	1	1	**1**	**0**
	Coronal	1	1	1	**1**	**0**
VIII	Apical	1.25	1.25	1	**1.1667**	**0.1443**
	Middle	1	1	1	**1**	**0**
	Coronal	1	1	1	**1**	**0**

Bio-mechanical preparation of the root canal produces a layer of organic and inorganic material that also contains microorganisms and their byproducts. Much of material is made up of small particles of mineralized collagen matrix and is spread over the entire surface of the cut dentinal surface to form what is called as the smear layer.

The identification of the smear layer was made possible using the electron microscope with the scanning electron microscope attachment and was first reported by Eick et al in 1970 [29] and they showed that the smear layer was made up of particles ranging in size from 0.5-1.5µm. They based their research on cut cavity surfaces of teeth. The smear layer produced in a cavity preparation on the coronal portion of the tooth structure and that produced during biomechanical preparation of the root canal may not be directly comparable. The reason being, the tools used for preparation are different and also the radicular dentin tubule numbers show a lot more variations with the likelihood of more soft tissue remnants being present.

Mc Comb and Smith (1975)[56] were the first to describe the smear layer on the surface of the instrumented root canals. They reported that the smear layer constituted of not only remnants of dentin as in the coronal smear layer but also remnants of odontoblastic processes, pulp tissue remnants and microorganisms. Though the thickness of the smear layer has been reported to be generally 1-2µm (Mader et al 1984)[51], they may vary depending on the type and sharpness of the cutting instruments, and whether dentin is dry or wet when being cut (Cameron in 1988)[18].

Cameron also observed that in early stages of instrumentation the smear layer on the walls of the root canals can have a relatively high organic content because of necrotic and or viable pulp tissue in the root canal space. The amount of smear layer produced during a motorized preparation is greater in volume when compared to hand filing (Czontkowsky et al in 1990)[21]. With the current trend in endodontics of routinely using rotary instruments to prepare root canals more volume of smear layer tends to be generated. The available endodontic literature indicates that these rotary systems will in most cases create a thick smear layer than manual instrumentation systems.

Smear layer has also been studied as two distinct components, the superficial layer and the deeper layer which is packed into the dentinal tubules for varying depths (40-110µm). Various mechanisms have been proposed for the penetration of the components of the smear layer into the tubules. The action of the burs and instruments, capillary action between the dentinal tubules and the smear material as a result of adhesive forces (capillary action hypothesis) possibly explain the tubular packing phenomenon.

Surface active agents have been shown to increase the depth of penetration of smear layer components into the tubules (Aktener et al in 1989)[1]. The available endodontic literature indicates that these rotary systems will in most cases create a thick smear layer than manual instrumentation. The generation of a smear layer is almost inevitable during a root canal preparation procedure. Though the smear layer was first reported almost three decades back, there is a lot of debate on the question of whether to keep it.

Some authors have suggested that keeping the smear layer may possibly block the dentinal tubules and limit the penetration of the tubules by the microorganisms or their byproducts by altering dentin permeability. In contrast other experts believe that the smear layer must be completely removed from the surface of the canal walls as it can harbour debris, microorganisms and can be detrimental to the effective disinfection of the dentinal tubules by preventing the irrigants and the intracanal medicaments from penetrating the dentinal tubules. They can also act as a barrier between the obturating materials and the canal wall and thus may interfere with the formation of an effective seal.

One of the primary factors affecting the prognosis of the root canal therapy is the failure to obtain an effective three dimensional seal of the root canal system. A number of studies have evaluated the role of the smear layer on achieving a satisfactory apical and coronal seal of the root canal space (Shahravan et al in 2007)[90].

There has also been a mid pathway concept of modifying the smear layer in such a way that it becomes completely resistant to dissolution or disintegration. This results in complete permanent blockage of the dentinal tubules in the root canal. Such an alteration of smear layer has been observed when treated with Titanium tetrafluoride (TiF_4). When observed under a scanning electron microscope the Titanium tetrafluoride treated root canal surface presented a massive and definitive surface coating blocking the tubules regardless of the presence or absence of the smear layer. (Sen and Buyukylimaz in 1998)[86]. The smeared surfaces showed a thicker coating

1-5µm than the unsmeared surfaces and also the commonly used root canal irrigants were not able to remove or reduce the thickness of this surface coating. The smeared dentinal surface when treated with Titanium tetrafluoride results in an acid stable and resistant state (Kazemi et al in 1999).[47] This finding has got a tremendous potential to reduce microleakage in endodontics and further studies with regards to the effect of Titanium tetrafluoride on periapical tissues, interaction with root canal irrigants and obturation materials have been recommended.

Removal of the smear layer significantly improves the apical seal and coronal seal of the obturated root canal space and this effort is not dependent on the type of obturation, site of leakage test, the type of sealer, the type of dye used for testing and the duration of the test as observed by Shahravan et al in their meta analysis in 2007.[90]

Various reasons have been put forward to support the idea of **smear layer removal**.

1. The presence of microorganisms and necrotic tissue.
2. The unpredictable diameter and volume (due to greater portion of it consisting of water).
3. A role possibly in acting as a bacterial substrate and letting them penetrate deeper into the dentinal tubules.
4. Preventing the penetration of irrigants and intracanal medicaments into the dentinal tubules.
5. Affecting the bond between the root canal sealer and the dentin.
6. Being a loosely adherent structure can contribute to microleakage.

Some authors who believe that the smear layer should **not be removed** have based their arguments on

1. It effectively blocks the dentinal tubules and therefore
2. Prevents inward or outward movement of microorganisms or other irritants and toxins.

William and Goldman in 1985 reported that the smear layer is not a complete barrier and can only delay the penetration of microorganisms, their by products and other toxins.

The smear layer removal seems to improve the fluid tight seal of the root canal system based on the various invitro studies performed and it seems reasonable to suggest that the smear layer should be removed.

With the introduction of resin based sealers and bonding systems for posts, the bond achieved between the dentin and the dentin adhesive is crucial to the achievement of an effective coronal and apical seal. Various studies have reported that the efficacy of dentin adhesives mostly depend on the smear layer removal and the formation of resin-dentin interdiffusion zone.

The hybridized smear layer produced by self etching adhesives is a weak area in the bonding interface as the top layer of the hybrid layer consists of disorganized collagen fibrils that degrade over time. Hence effective removal of the smear layer from the dentinal surface of the root canal becomes necessary for allowing the infiltration of a self etching adhesive. Smear layer removal has been shown to enhance the adaptability of gutta percha (both cone and thermo-plasticized) and resin based sealers.

The concept of a root canal preparation without the removal of a smear layer has also been visualized. A non-instrumental hydrodynamic technique by Lussi et al in 1993 [49] and a hydrodynamic disinfection technique by Ruddle CJ in 2007 [77] in which sonically driven polymer instruments with tips of variable diameter have been proposed.

Smear layer removal has been shown to alter the diffusion permeability of the radicular dentinal surface as reported by Galvan et al in 1994.[32] The permeability was reduced immediately after removal of smear layer contrary to the thought that the removal of smear layer opens up the dentinal tubules and would thereby increase permeability. But the permeability increased slowly over a period of two months. They postulated that probably the methodologies created precipitates deep within the tubules which reduced permeability initially but as they dissolved the permeability increased. During endodontic therapy there is a potential that various materials and medicaments which are kept within the root canal space can penetrate and pass through the dentinal tubules to the periodontium and can affect the periodontal status adversely. The diffusion of the medicament into the tubules depends on the diffusion properties of the medicament and not just on the permeability of the dentinal tubules.

Over the years various authors have advocated different methodologies for efficient smear layer removal. Chemical, ultrasonics, LASERS and more recently sonic techniques have been used either alone or in combination.

These irrigant solutions ideally should be able to remove the organic components of the smear layer and the mostly inorganic components of the smear layer. No single solution offers the above capabilities and therefore multiple irrigant combinations and techniques have been advocated for successful smear layer removal. The concept of a working solution and an irrigant solution was put forward by Kaufmann in 1986 [46] where the working solution was the one which was first used to clean the canal and the irrigant solution was the one which was essential to remove the debris and smear layer created by the instrumentation process. Additionally of late irrigant solutions with antibacterial properties and substantivity through adherence to radicular dentin have been tried.

Various attempts at new formulations have been made to create an ideal irrigant solution which combines the beneficial effects of all individual components. Sodium hypochlorite has excellent tissue dissolving action which increases with rise in temperature upto 60ºc but its ability to remove smear layer from instrumented canal walls has been found lacking.

Chlorine dioxide (ClO_2) is similar to sodium hypochlorite and was found to be as efficient as sodium hypochlorite in organic tissue dissolution. Chlorine dioxide produces little or no trihalomethanes when compared to sodium hypochlorite. Trihalomethane is an animal carcinogen and a suspected human carcinogen. Chlorine dioxide therefore might be a better alternative than sodium hypochlorite. (Levesque et al in 2002)

Various chelating agents have been tried as root canal irrigants, EDTA (Ethylene Diamine Tetra Acetic acid) being the most popular in the concentration of 17%. They are efficient in removing the inorganic component of the smear layer and radicular dentin surface by chelation. Peroxides and surfactants have been added to EDTA to increase the effectiveness of debris and smear layer removal. The surfactants reduce the surface tension and help EDTA to effectively penetrate the dentinal tubules. They are also available in paste and gel form which are used as canal lubricants during the instrumentation process. The liquid form has been most effective at smear layer removal.

EDTAC a combination of EDTA and Cetavlon, REDTA, EGTA (Ethylene Glycol Tetra Acetic acid) have been tried. A 2%EDTA and a surface active antibacterial agent BDA (Bis Dequalinium Acetate) was found to be very effective with no erosion of the peritubular and intertubular dentin even in the apical one thirds.

Tetracyclines including Tetracycline hydrochloride, Minocycline and Doxycycline in addition to their antibacterial properties, have at low pH values an ability to act as calcium chelators and cause enamel and root surface demineralization. (Bjorvatn in 1982).[12] Barkhordar et al in 1997 [9] reported that doxycycline in a concentration of 100mg/ml was effective in removing the smear layer from the surface of the instrumented canals and also speculated a reservoir of antibacterial agent remaining within the tubules for a period of time.

Organic acids have also been tried as root canal irrigants aimed at removal of smear layer and have been found to be effective. A 10% solution of citric acid solution was particularly effective. Citric acid was found to leave precipitated crystals in the root canal which might be disadvantageous during obturation. 50% lactic acid, 25% tannic acid were also tried. Tannic acid was found to increase the cross linking of the exposed collagen with the smear layer and within the matrix of underlying dentine, and therefore increasing organic cohesion to the tubules. Poly acrylic acid has also been tried and found to be very effective in concentration of 10-40%. Because of its potency an exposure time of not more than 30seconds was recommended. (New Berry et al in 1987).[66] A 7% Maleic acid solution which is used as a conditioner in adhesive dentistry has been shown to be more effective than 17% EDTA in smear layer removal from root canals.

Peroxides as solutions have also been tried but were more effective in debris removal and posed potential risks when they reached the peri-apical region even in small quantities.

As investigators tried out newer methodologies they found combinations of irrigants to be most effective at smear layer removal, as per the concept of a working solution and a irrigant solution put forward by Kaufmann and Greensberg in 1986.[46]

The combination of sodium hypochlorite and EDTA was found to be particularly effective at smear layer and debris removal. As there was no single solution which had the ability to dissolve the organic tissues and demineralize the inorganic layer the sequential use of organic and inorganic solvents was advocated (Baumgartner in 1984)[10]. A 5% sodium hypochlorite solution and 17% EDTA solution were found to be most effective in combination. Etidronic acid (HEBP:1-Hydroxyethylidene-1,1-bisphosphonate) does not react with sodium hypochlorite in short term and is a potential alternative to EDTA or citric acid and is also non-toxic.

A 0.5% solution of salvizol 0.5%BDA (Bis Dequalinium Acetate) was shown by Kaufmann et al in 1978 to be effective at chelation and removal of organic debris when compared with 5% sodium hypochlorite both were found to be comparable in their ability to remove organic debris but only 0.5%BDA (Bis Dequalinium Acetate) opened up the dentinal tubules.

The chemo-mechanical action of sodium hypochlorite removes the loosely attached debris and organic material while chelating action of EDTA effectively removes the inorganic part of the smear layer. Various combinations of sodium hypochlorite and other chelating agents have been tried. Various other methodologies have also been used to enhance smear layer, debris and microorganisms from within the canal systems.

LASERS can vaporize tissues in the main canal, remove microorganisms and eliminate residual tissue in the apical portion of the root canals. The main issues with the laser systems in the smear layer removal are the access to the small canal spaces in the periapical region and the relatively large probes that are available. LASER activation of the irrigant was found to be effective in smear layer removal (Peeters and Suardita in 2011)[70]. They attributed removal of the smear layer to cavitation which is the formation of a vapour or a cavity that contains bubbles inside a fluid. These bubbles expand 1600 times their volume which allows the irrigants to access the apical portion of the canal more readily and in addition these bubbles become unstable and collapse what is called as an implosion resulting in a shock wave. These LASERS generated waves move at high speeds and appears to enhance the action of the irrigants of the irrigants. Application of the LASER is done via., a fibre tip and this technique of irrigant activation appears promising in the apical thirds of the canals with closed apices.

Ultrasonics were used in conjunction with sodium hypochlorite for the removal of smear, debris and microorganisms from within the root canal. Cavitation and acoustic streaming of the irrigant due to ultrasonic activation was found to be beneficial.

As the concept of final rinse solution gained popularity the improvement of these solutions to enhance antimicrobial, smear and debris removal was done.

Removal of microorganisms from within the canal space and that which survive in biofilms and within the dentinal tubules have been a major concern during endodontic procedures. The ability of these organisms to survive and cause recurrent infections has necessitated means and mechanisms for their elimination and removal from within the root canals. Antibacterial properties of the irrigants have thus become a necessity. As these organisms tend to survive for periods of time in a dormant state irrigants with antibacterial components that can bind to the dentinal structure and be released over a period of time (substantivity) they have become popular. Bacterial contamination of the root canal can also occur:

1. During endodontic procedures when rubber dam is not used.
2. Due to leakage during temporization between visits.
3. Reinfection due to the growth of microorganisms that may have survived endodontic treatment procedures.
4. Coronal or apical microleakage.

Sodium hypochlorite possess a potent antimicrobial action however it does not possess any residual antimicrobial activity. Calcium hydroxide which is used as an antimicrobial intracanal medicament dressing does not have residual antimicrobial activity.

Chlorhexidine and tetracycline have been found to possess the property of substantivity. The duration of exposure, irrigation volume, and concentration seem to play a role in the duration of the residual antimicrobial efficacy. A 2% chlorhexidine solution has been found to be very effective as a root canal irrigant with good antimicrobial properties, and residual antimicrobial activity. The positively charged ions of chlorhexidine absorb onto the dentin and prevent bacterial colonization of the dentinal surface, some time beyond the actual period of medication. Tetracycline also readily attaches to dentin and are subsequently released without losing their antibacterial efficacy.

A 3.8% W/V solution of Silver diamine has been developed for intracanal irrigation, which was found to be bactericidal. Silver deposits were found to occlude the tubules after removal of the smear layer. Triclosan and Gantrez have been tried as root canal irrigants and are bactericidal against endodontic pathogens. The Minimum Bactericidal Concentration (MBC) was 10.4µg/ml. Various herbal preparations have been tried as endodontic irrigants. Triphal an extract from medicinal plants (Terminalia Bellerica, Terminalia Chebeula and Emblica Officinalis) is bactericidal and its fruits contain citric acid and which might be effective in smear layer removal. (Prabhakar in 2010).[72]

Green tea extract (Camelia Siensis) which contains polyphenols have shown to be antibacterial against E.Faecalis biofilms. Morinda citrifolia has also been tried as a root canal irrigant and has been shown to be very effective in smear layer removal as similar to 6% sodium hypochlorite in combination with EDTA. This might present advantages as sodium hypochlorite accidents could be avoided as Morinda Citrifolia extract is a biocompatible antioxidant. (Murray et al in 2008).[62]

Attempts to develop an irrigant with multiple desirable properties have led to the development of newer irrigant formulations like MTAD. Biopure MTAD (Dentsply, Tennessee USA) was introduced by Torabinejad in 2003[101]. This was a mixture of 3% doxycycline, 4.25% citric acid and a detergent (0.5% polysorbate 80). Several studies have evaluated the effectiveness of MTAD in removal of smear layer and antibacterial action.

The tetracycline component provided the antibacterial action. Citric acid helped in smear layer removal and allowed tetracycline to enter the dentinal tubules, binding to dentin and release over a period of time without losing its antibacterial activity (substantivity). The detergent component reduces the surface tension and improves penetration within the dentinal tubules. The antibacterial effect of MTAD may be inhibited by buffering effect of dentin and serum albumin present in the canals.

MTAD has been recommended for use as a final rinse solution for five minute exposure time with a volume of 5ml. The recommended initial rinse solution is 1.3% sodium hypochlorite solution during instrumentation. Sodium hypochlorite is needed as an irrigant to assist MTAD to completely remove the smear layer. A higher concentration of sodium hypochlorite at 5% is much more effective at dissolution of organic debris and remnants and is much more toxic. As there was no significant difference between the ability of 1.3%, 2.6% and 5.25% sodium hypochlorite as initial rinses with MTAD as a final rinse in smear layer removal, it seems prudent to use the lowest concentration of sodium hypochlorite (1.3%) during instrumentation. (Torabinejad in 2003)[102].

When MTAD is applied to 1.3% Sodium hypochlorite irrigated dentin a photosensitive discoloration reaction occurs, a phenomenon which is caused by the oxidation of MTAD by sodium hypochlorite similar to peroxidation of tetracycline by reactive oxygen species. The reaction is exothermic. The dentin bound yellow precipitate requires light to convert to dark brown. This causes a potential iatrogenic staining and may be seen when teeth prepared for veneers. This type of reaction could be prevented by flushing with distilled water after use of sodium hypochlorite and subsequently using reducing agents like 10% ascorbic acid and or glutathione which neutralizes the oxidative potential of sodium hypochlorite and also prevents the photo degradation of doxycycline.(Tay et al in 2006).[95,96]

A reduction in the antimicrobial substantivity of MTAD has also been observed when used with 1.3%sodium hypochlorite as an initial rinse.

Another alternative would be to try other solutions which are as effective as sodium hypochlorite in organic tissue dissolution but cause less extrinsic staining by photo sensitive reaction with doxycycline. The genotoxic damage caused by Biopure MTAD was evaluated and found to cause genetic damage in-vitro. As DNA damage is an important step leading in events leading from carcinogen exposure to cancer, exposure to these compounds should be evaluated with potential health risks.(Marins et al 2009)[53]

Tetraclean is another doxycycline based irrigating solution with a lesser concentration of doxycycline and acid and a detergent (polypropylene glycol). It is highly microbicidal against biofilms when used as a final rinse for five minutes, when compared to MTAD. Compared to MTAD tetraclean has got a low surface tension. The low surface tension of both these irrigants increases their surface area of intimate contact with the dentinal walls and may permit deeper penetration into the dentinal tubules enabling better antibacterial action. (Giardino et al in 2006)[36, 37]

Current concepts of biomechanical preparation indicate application of chemicals to instrumented root canal surfaces for smear layer removal. Various studies have shown that the endodontic irrigants are capable of altering the chemical composition of dentin by removal of major inorganic elements such as calcium ions (Ca^{2+}) present in hydroxyapatite. The changes in the Ca^{2+} ratio might change the microhardness, permeability and solubility characters of dentin. It might also affect the bonding of the resin based root canal sealers as it depends on the presence of residual Ca^{2+} ions in the bonding area reduces their bond strength. Chelating acids and acids decalcify dentin depending on application time, pH value and concentrations. Though it was thought that sodium hypochlorite removes only magnesium and carbonate ions, recently it has been shown to remove Ca^{2+} ions. On a study which compared the various irrigant solutions with regard to their decalcifying effects, MTAD was found to be least decalcifying.

Ozonated water has also been tried as a root canal irrigant. Electrochemically activated water (ECA) has also been tried as a root canal irrigant and has been shown to be microbicidal against a variety of organisms and have been used routinely for sterilization of the dental unit water lines, endoscopes, instruments, disinfection of chronic wounds, etc. Use of this water as a root canal irrigant was evaluated by Quing et al in 2006 [73] and they observed that with ultrasonication this resulted in smear layer removal and did not affect the micro hardness of the dentin. The active component of ECA is hypochlorous acid (HOCl). Hypochlorous acid is produced by body's immune cells via., a chain of aerobic reactions called the oxidative burst pathway to kill invading pathogens and to fight infection. Hypochlorous acid is produced by electrochemically charging a low concentration of salt solution using an element reactor. Hypochlorous acid is biocompatible and antimicrobial against a wide variety of microorganisms.

The Electrochemically Activated Water used in this study was STERILOX [R] at a concentration of 144mg/l of chlorine. The disinfectant is generated by passing the saline solution over coated titanium electrodes at 9amps. The product has a pH of 5.0-6.5 and oxidation reduction potential (Redox) of >950Mv. This is non-corrosive, non-damaging to dental equipment and has been found to be biocompatible. Extensive tests of Sterilox technologies U.K using international tests for exposure and toxicology have failed to show any harmful effects. It needs to be freshly prepared and is effective for 48hours.

Solovyewa and Dummer in 2000 [94] have concluded that electrochemically activated solutions were effective in cleaning root canal walls and may be a potent alternative to sodium hypochlorite as a root canal irrigant during endodontic therapy.

In the present study Electrochemically Activated Water(STERILOX ^{R}) was evaluated as a root canal irrigant for removal of smear layer and debris, erosion of the canal walls. It was tried in different groups using different protocols as initial and final rinses. The control groups used were Saline (Group I) and 5% sodium hypochlorite + 17% EDTA (Group II).This was compared with Biopure MTAD which was used as per the manufacturer's recommendations, and NaOCl (Sodium hypochlorite).

Sodium hypochlorite is very toxic, has caustic hazard, risk of emphysema when pushed beyond the confines of the root canal and causes allergic reactions. The wide spread use of this material is explained by its low price, excellent tissue solvent action, and its root canal disinfection properties.

The results of the present study indicate that Sterilox is as effective as sodium hypochlorite in smear layer removal when used with 17% EDTA as a final rinse (Chart-2). When analysed statistically there is no significant difference between the two groups **($p > 0.05$)** using **Mann-Whitney U test** and **Student's - t** tests for a **p-value of 0.005**.

Complete removal of vital and necrotic remnants of pulp tissue, microbes and their by-products, smear and debris are essential for successful endodontic therapy. The rotary instruments which are currently widely used only act in the central body of the canal leaving isthumi, cul-de sacs untouched after the completion of the preparation. These areas harbour unwanted material and prevent close adaptation of the obturating material. The role of the irrigant is necessary to achieve cleaning in these areas. Increasing the temperatures, addition of surfactants, increase the efficacy of irrigants. Dual use of irrigants is commonly followed to complement the short comings that are associated with a single irrigant. The irrigants must be brought into contact with the entire canal walls to be effective.

Irrigant delivery is an important parameter and of date various methods of irrigant delivery have been tried. This study was performed with a side vented needle with the vent at 1mm from the tip. Computational dynamic fluid flow studies have demonstrated the limitations of a side vent delivery on irrigant replacement and appropriate modifications were made in this study protocols to enhance irrigant replacement. The volume of the irrigant also plays a crucial role and a volume of 10ml during the initial rinse and 5ml during the final rinse was adapted in the protocol. The duration of the exposure of the final rinse also played a role in the efficacy of the final rinse providing sufficient time for action of the irrigant.

To further improve the action of the irrigants various agitation techniques have been developed which are either manual or machine assisted systems. Passive irrigation systems have a lot of short comings in irrigant delivery. Manual dynamic activation where a well fitting gutta percha point is placed to working length and moved up and down in 2-3mm strokes can sufficiently improve the displacement and exchange of the irrigant.

This system has been found to be very effective. In this study this subgroup was comparable to sonic and ultrasonic subgroups over the groups compared.

Machine activated systems are very popular as they are aggressively promoted, are fast and save time. Brushes (motorised), sonic and ultrasonic systems (Passive Ultrasonic activation and continuous), pressure alternation devices have been introduced. But there is no evidence based studies which correlate the efficacy of these devices with improved treatment outcomes. Vapour lock effect results from the reaction of the irrigant with the debris and smear forming bubbles, forming close ended microchannels, which take a very long time to flood back with the irrigant. A simple method to disrupt the vapour lock effect would be to insert a file or gutta percha which is well fitting to the prepared canal to working length after instrumentation. Acoustic streaming and cavitation becomes impossible after a vapour lock. The subgroups with Passive ultrasonic activation were found to be effective than sonic at the apical one third and the sonic was more effective at the coronal one third. The proper activation of these systems after removal of a vapour lock is necessary especially in a clinical setting. This study adopted a closed ended root canal system as it is compared well with the natural conditions present in the oral cavity.

On a comparison between the Groups IV and VII where Sterilox^{R} was used as an initial rinse and compared with manufacturers protocol for MTAD, there was no significant difference when statistically analysed using the **Mann-Whitney U test** and **Student's - t** tests for a **p-value of 0.005**. STERILOX^{R} has the potential to be used as an initial rinse during the endodontic procedures in place of sodium hypochlorite. In addition this has got other excellent properties of biocompatibility, operator safety and safe when extruded periapically. This is also effective against a variety of micro organisms though the antimicrobial efficacy has been reported to decrease with increased organic contaminants which could be effectively overcome with sufficient irrigant volume and replacement. Further analysis of data also indicate that it is not effective as a final rinse solution when compared with MTAD and EDTA and a significant difference was found between the groups when analysed statistically. **($p < 0.05$)**

With emerging regenerative endodontic therapies there is a look out for biocompatible irrigants and Electrochemically Activated Water (STERILOX^{R}) would possibly be a better alternative to sodium hypochlorite in this regard.

One hundred and fifty teeth were collected cleaned and stored in normal saline. They were investigated for the presence of a single canal and standardized to a length of 15mm by sectioning at the level of the cemento-enamel junction.

The teeth were then embedded in polyvinyl siloxane material after sealing the apices with wax and subsequently divided into control (n=5) and experimental groups (n=12). A total of eighty two teeth were used for the purpose of the study.

The root canals were prepared using the Protaper system with X-Smart endomotor with 1:16 reduction handpiece with protocols for irrigant rinse during instrumentation and final rinsing. Electrochemically activated water (STERILOX^{R}) and Biopure MTAD were evaluated and compared for their effect on smear layer and debris removal in the apical, middle and coronal one thirds using a scanning electron microscope. The images were analysed, results tabulated and statistically analysed

On conclusion of the study on the evaluation of comparative efficacy of smear layer and erosion of three irrigating solutions on intraradicular dentin using Scanning Electron Microscopy the following conclusions are made

- Overall the apical one third presented the most amounts of smear and debris among all groups with the mean values of **3.025** and **2.075** respectively.
- Among all the groups observed for the effect of erosion all the thirds of root canal exhibited erosion ie., loss of peritubular dentin and intertubular dentin with Group II presenting the highest erosion in the coronal and middle thirds and in apical one thirds both Group II and Group VIII presented similar levels of erosion.
- Electrochemically Activated water (STERILOX^{R}) was efficient at removal of smear layer and debris and results comparable to that achieved by sodium hypochlorite. On statistical analysis there was no significant difference between Groups IV and VII.
- Electrochemically Activated water (STERILOX^{R}) is recommended for use as an initial rinse and is as effective as sodium hypochlorite and offers a huge potential as it is highly biocompatible.
- Electrochemically Activated water (STERILOX^{R}) is not recommended for use as a final rinse solution.

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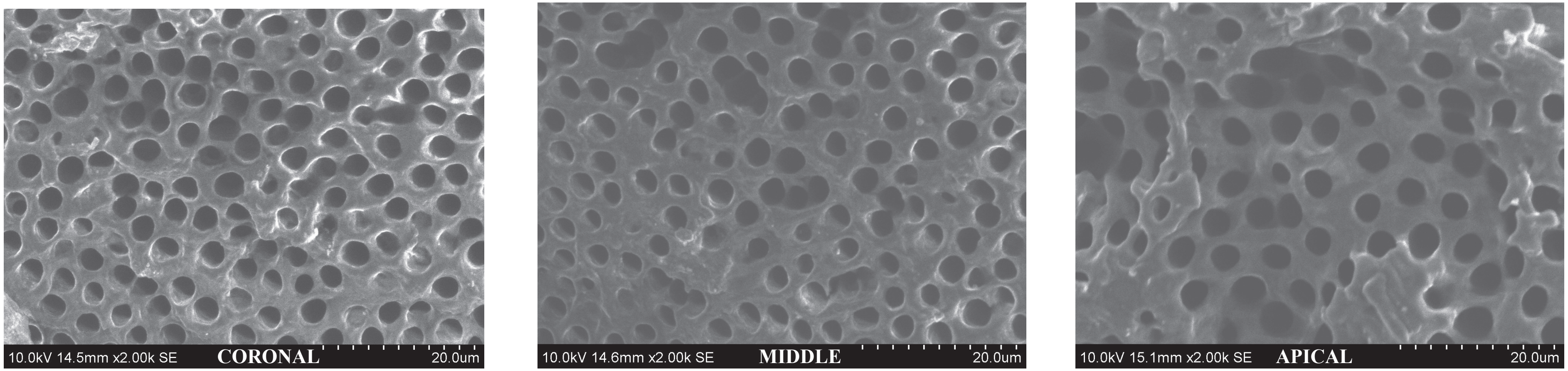
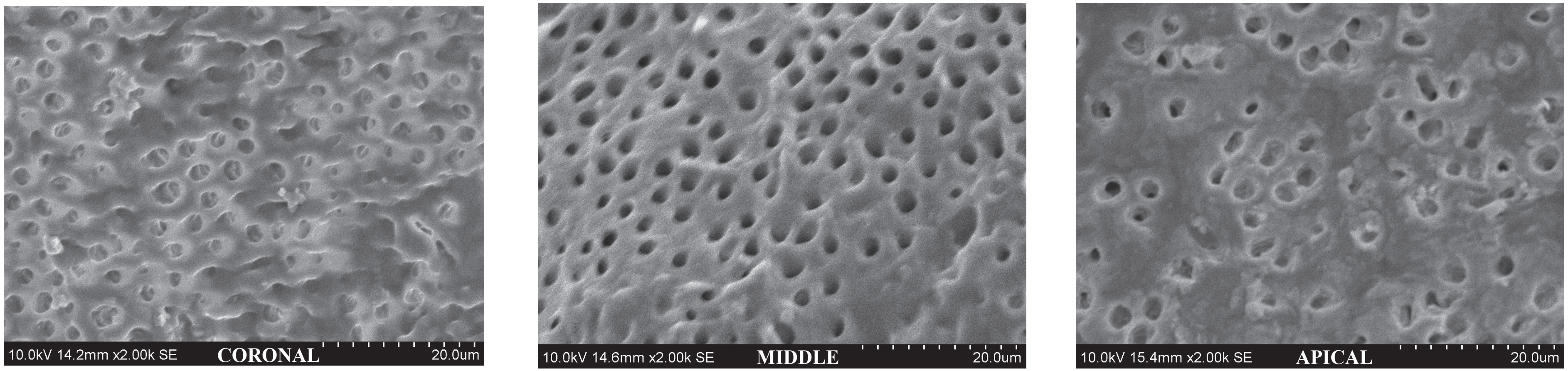
SEM Image Comparison of Groups II & III

Group - II (5% NaOCl + 17% EDTA)

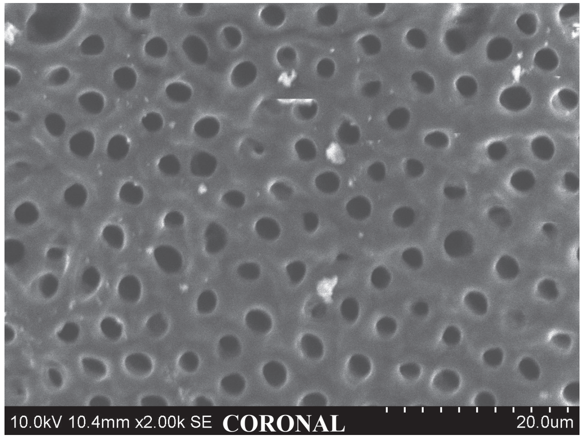


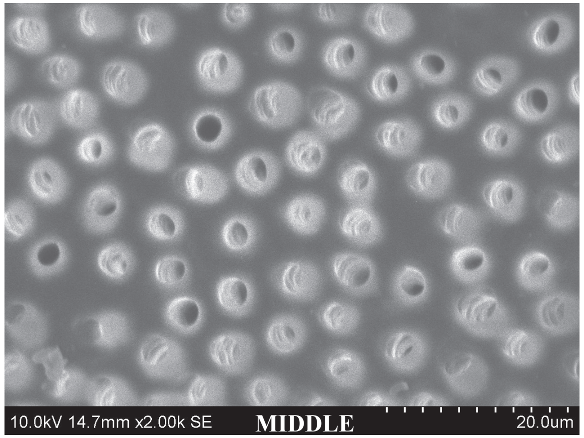
Group - III (STERILOX + 17% EDTA)

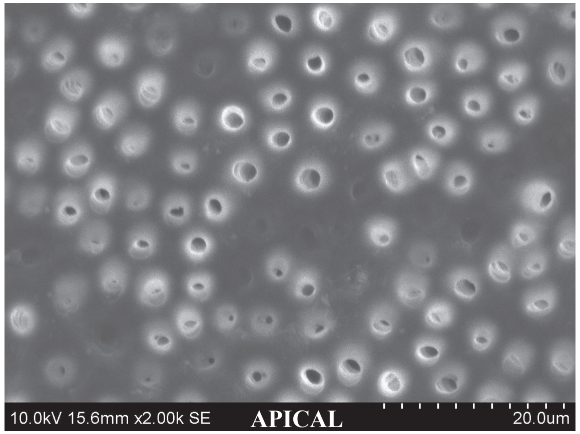


SEM Image Comparison of Groups IV& VII

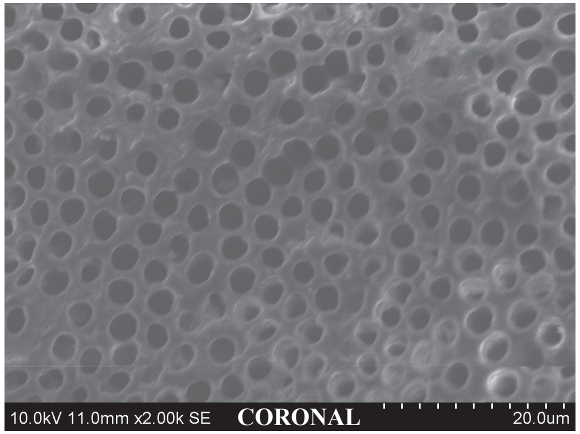
Group - IV (STERILOX + Biopure MTAD)

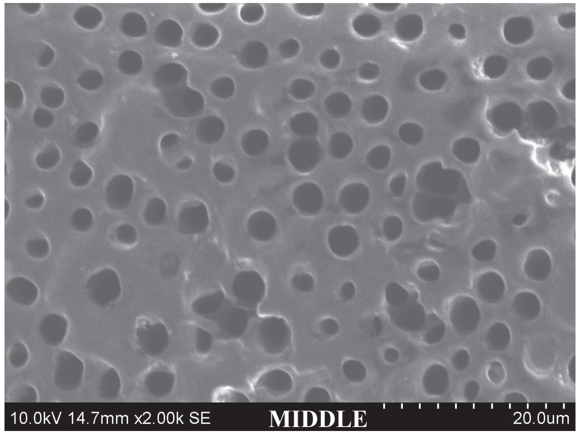


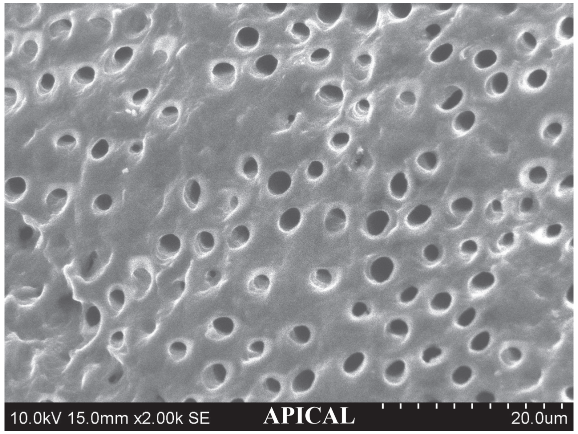




Group - VII (1.3%NaOCl + Biopure MTAD)

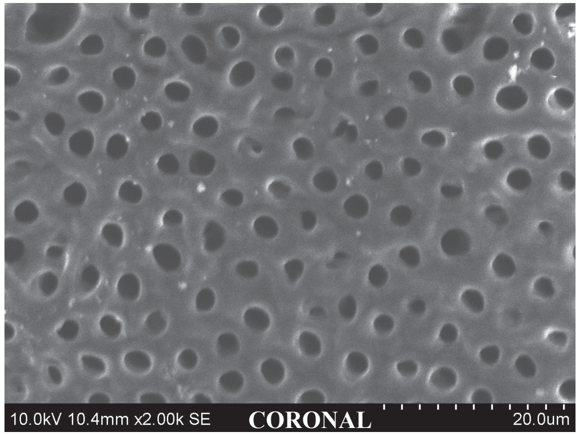


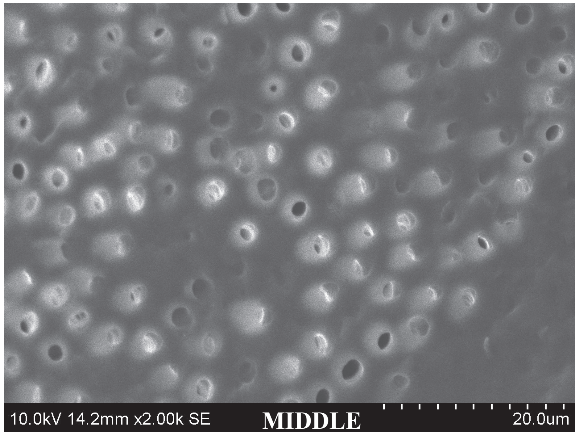


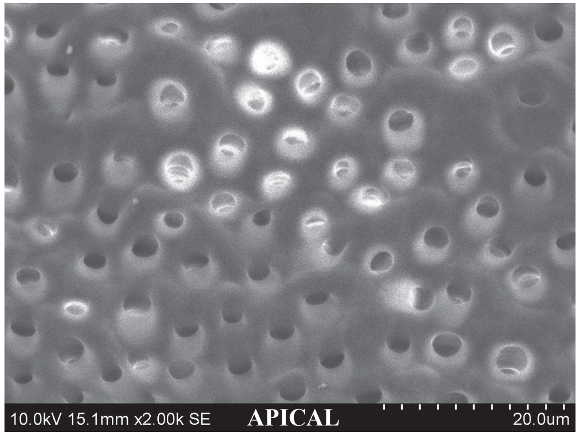


SEM Image Comparison of Groups IV & VIII

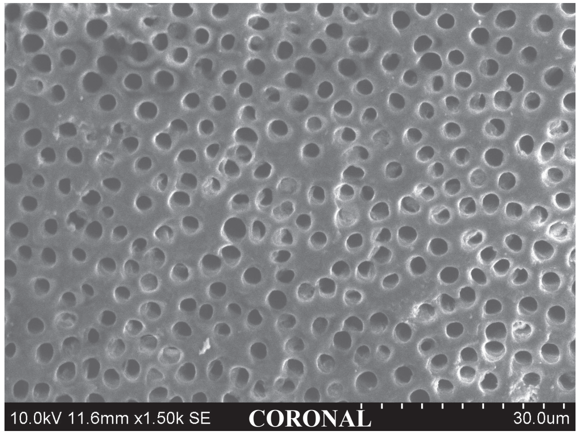
Group - IV (STERILOX + Biopure MTAD)

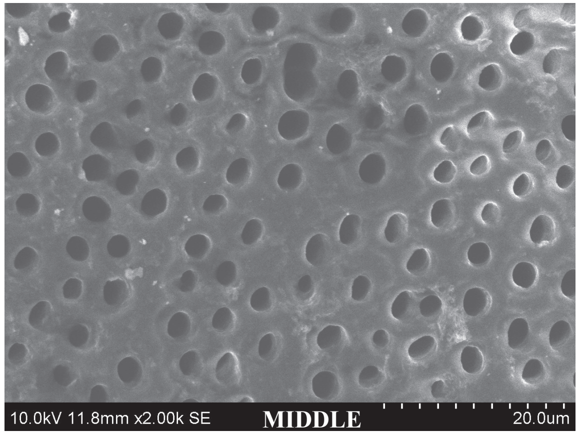


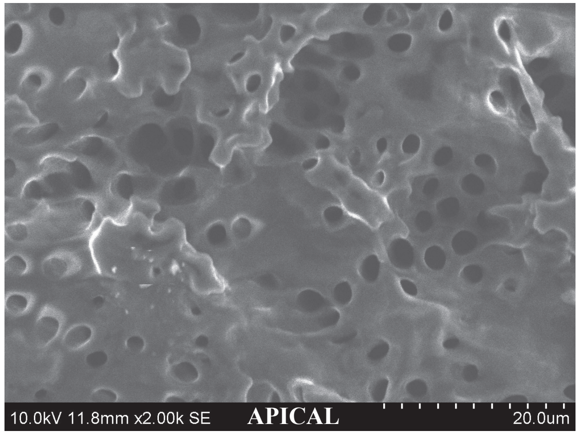




Group - VIII (5 %NaOCl + Biopure MTAD)

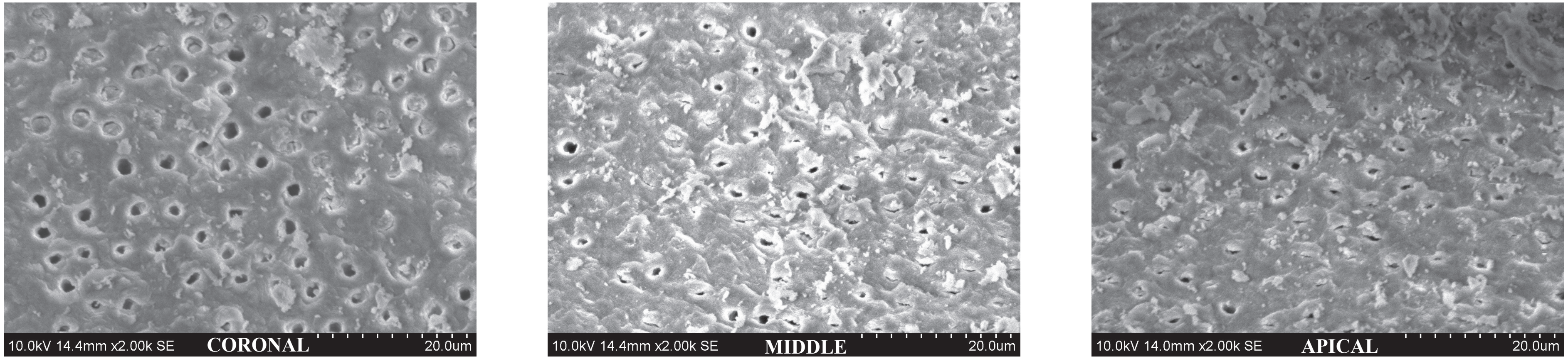




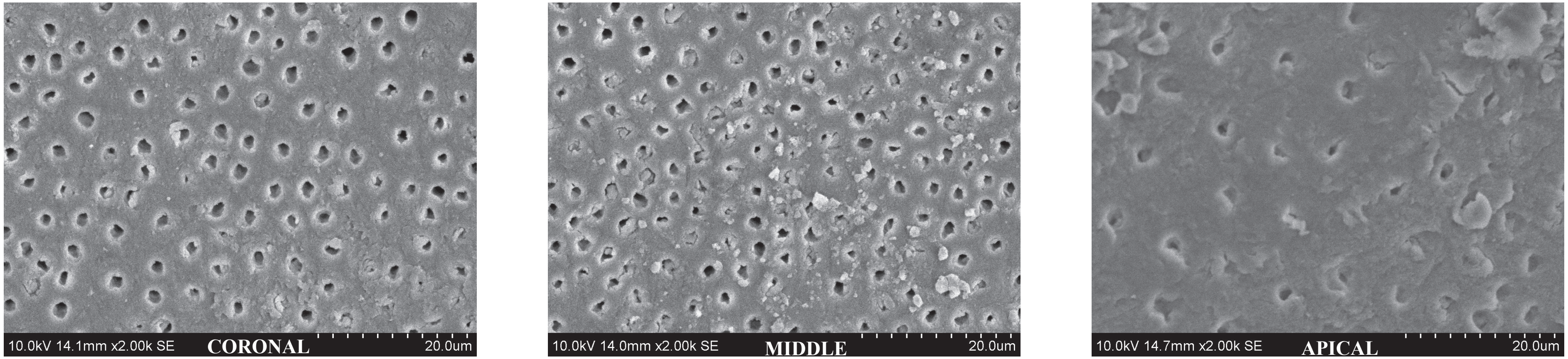


SEM Images of Groups V& VI

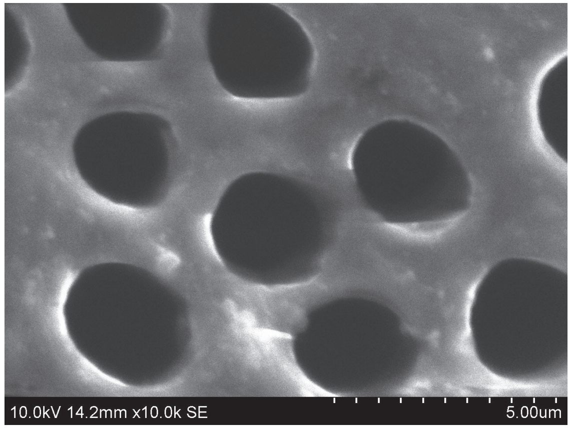
Group - V (1.3%NaOCl + STERILOX)



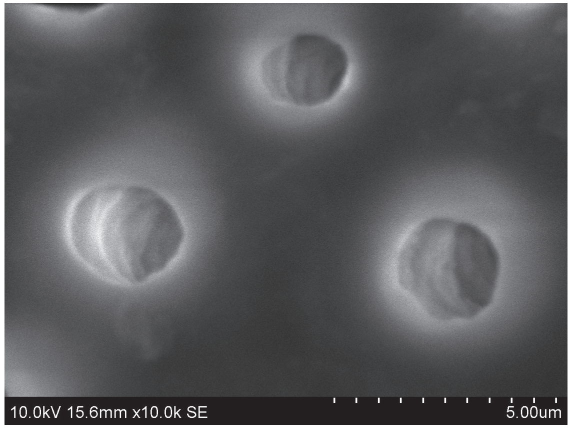
Group - VI (5 %NaOCl + STERILOX)



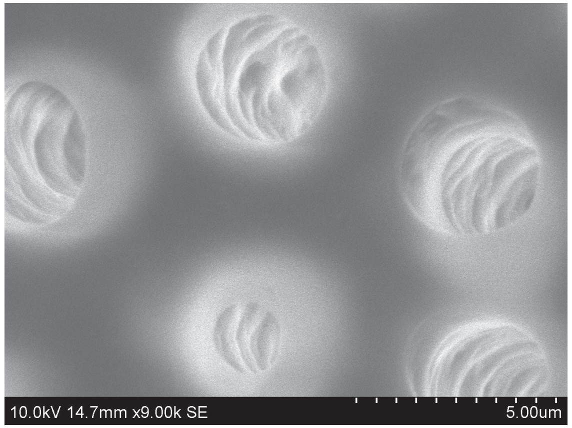
SEM Images at X10,000 Magnification



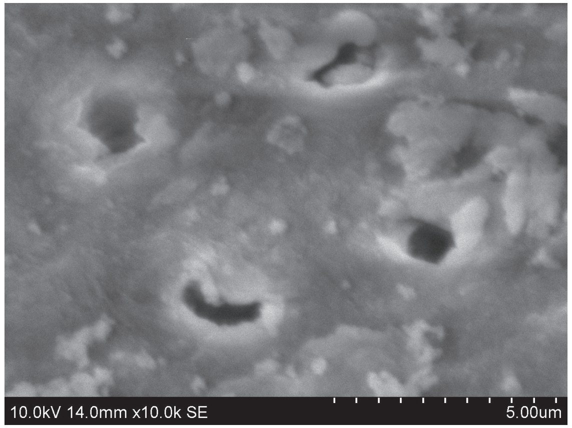
Group-VII 1.3% NaOCl + MTAD
Clean dentinal tubules devoid of dentinal plugs



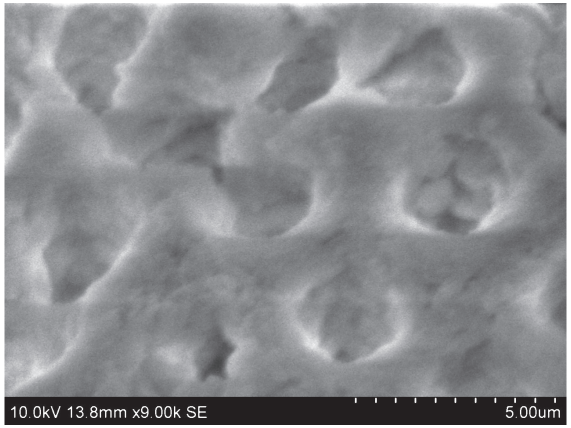
Group-IV STERILOX + MTAD
Clean dentinal tubules devoid of dentinal plugs



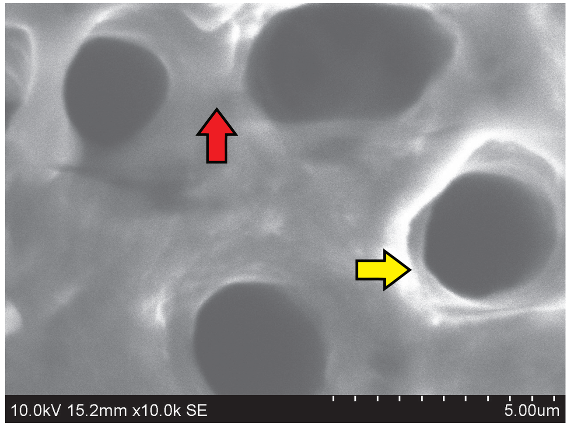
Group-IV STERILOX + MTAD
Clean dentinal tubules with collagen fibres



Group-V Dentinal tubules partially blocked with Smear layer , scattered Debris seen



Group-VI Dentinal tubules blocked with dentinal plugs



Loss of Intertubular dentin
Loss of Peritubular dentin